

Can the Piper Play this Tune?

SOME lame ducks come and some lame ducks go, but International Computers Limited is still with us. Exactly a year after the British government announced that it was lending the company £14.2 million in order that the impetus of its research and development programme be continued, Mr. Christopher Chataway with all the munificence characteristic of the present Conservative Administration, announced last week that the Government was again dipping its hand into its pocket to help the company. This time the scientists working in research and development with ICL are assured of support until 1976. The amount now offered is £25.8 million, or £8.6 million a year which, according to Mr Chataway, is of the "launching aid type" and is designed to ensure that ICL's work on this new range of computers, the first announcements of which are expected within a year, will continue.

Apart from the obvious question of whether in the long run such support does much good for the company, the critical question to be asked at this time is whether the British Government is sensible in providing massive support for a national computer company. Will ICL, or any other national computer company for that matter, ever be able to compete with the giants of the industry from the United States on their own terms even with massive government aid? The Commissioners of the European Economic Community, being realists for once, are in favour of mergers of national computing interests in order that Europe should have a strong computing industry. Mr Christopher Layton, Director for Advance Technological Industries at the EEC, a few months ago called for ICL to find a partner within Europe so that a sister consortium to the Siemens-Philips-CII unit would be available. But ICL has so far stubbornly rejected the entreaties of the EEC and the company, at present at least, seems to have no inclination for an association with any other company either inside or outside Europe. The government by continuously providing financial loans are indeed fostering the national rather than the international approach.

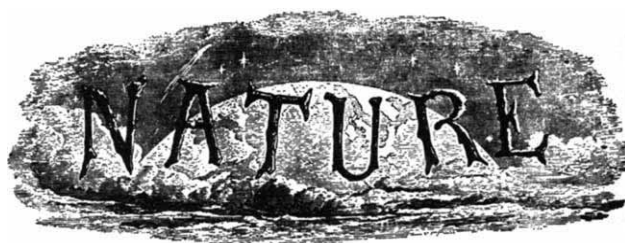
ICL, however, sees its future as being extremely rosy. In spite of the fact that the company's pre-tax profits have never exceeded 6% of the turnover, the Government is now convinced, after analysing the basis for ICL's growth predictions, that its total loan of £40 million will be repaid between 1977 and 1984 from profits that the company will make in excess of 7.5% of turnover. Not only is the company confident of its profit levels in the second half of the 1970s and early 1980s but it also stated that after 1976 it will not need any further financial assistance from the government.

On what is such confidence based? The computer industry is certainly booming at present but the lessons of the past few years should have taught everyone that periods of growth do come to an end—certainly 10 years is a very long time indeed to hope for prosperity in the computer industry to continue growing unabated. ICL is, of course, on the verge of announcing a new line of com-

puters and part of the confidence ensuing from the company must be based on the hope that these computers will sweep the market, but it remains to be seen whether it will be an effective counter to lines produced by other manufacturers.

In view of the bubbling self-confidence of ICL it is no surprise that it has so far turned up its nose at the suggestion of an association with another European company although it is quick to point out that it is in constant touch with other computer manufacturers and that if a technological rather than a political link would seem expedient then it would be given all consideration. But ICL's confidence contrasts sharply with the feelings of the European Commission on the future of computer companies within Europe. It is far from clear whether even two large multi-national European companies could compete effectively with IBM, and even a single large European company would perhaps not be an effective foil to the American giants in the field. But in spite of this ICL is going it alone. Such courage deserves commendation and it is only to be hoped that all goes well. But the British tax payer is going to have to wait a long time indeed to see whether or not he is going to get his money back.

100 Years Ago



Curious Rainbow

AN unusual atmospherical effect was witnessed here to-day, which I had a good opportunity of observing. The sun was about 8° from the horizon, shining brightly upon a heavy shower which had a background of dark clouds. The result was, of course, a double rainbow of remarkable brilliancy. In addition, however, to the ordinary circular and concentric bows, there was a third of an elliptical form, the two ends of which respectively sprang from the two ends of the inner arc, while the elliptical curve cut the outer arc at each extremity of a chord, which was parallel to, and which intersected the normal radius at a point about two-thirds of its length above, the diameter that formed the common base. The top of the elliptical bow was thus the outermost of the three, but the space between its inner margin and the outer margin of the second bow, although quite distinct, was not large.

The appearance of the third bow was due to light reflected from the sea. The sun being low, the resulting line of reflection was long, and it was the linear character of the source of light which gave the elliptical form to the bow it occasioned.

Dunskait, Ross-shire, July 10

GEORGE J. ROMANES

From *Nature* 8, 224, July 17, 1873.



OLD WORLD

Select Committee backs Tracked Hovercraft Research

THE Select Committee on Science and Technology has asked the government to open a centre for research on non-contact high speed transport technology at Earith based around the remains of Tracked Hovercraft Limited.

The committee's request was put to the government at the start of this week's hearing with Mr Michael Heseltine, Minister for Aerospace present. Before Mr Heseltine gave evidence, Mr Airey Neave, chairman of the Select Committee asked that the government accept the proposals formulated by Imperial College for a research centre at Earith (see *Nature*, 242, 490; 1973), that it keep the track open until the centre is ready, that it complete the extension of the test track, and that it provide a direct annual grant to cover the costs of a small permanent staff to run the site.

That Mr Heseltine was not taken entirely aback by these proposals was due to the fact that Mr Neave had warned him that they were coming, and his reaction was to point out that a proposal for a transport research centre was a matter for Mr John Peyton, Minister for Transport Industries, rather than for the Department of Trade and Industry.

Mr Heseltine did, however, agree to pass the proposals on to Mr Peyton. He also assured the select committee that although plans to dismantle the existing track were to be put into effect shortly, actual demolition is unlikely to start before October 1.

The Select Committee, having declared its colours, pointed out that maintaining the track cost less than £4,000 a month, while demolishing it was going to cost £250,000. Waxing lyrical over tracked hovercraft, as over some lost son, the select committee described the concept as "the most important transportation development in this country since the development of the jet engine" and told Mr Heseltine that all three of the companies in Britain which have an interest in linear motors—GEC, Hawker Siddeley and Linear Motors Limited—have said that they would use the site if it was available.

Mr Heseltine countered by pointing out that while all three would use it, none of them had said that the site was essential. None of them had offered to contribute to the running costs. If they said that they needed the track and were prepared to put up some money, Mr Heseltine said, then "that would be a consideration". It was five months now, Mr Heseltine said, since the closure of Tracked Hovercraft was announced,

and no proposal had been made. Companies still had until October 1 to act.

Persuading industry to become involved in the linear motor work had been no easy task Mr Heseltine said. Neither GEC and Hawker Siddeley with whom the government has had talks are prepared to put much of their own money into linear motors.

Mr Heseltine also emphasised that the Tracked Hovercraft facilities were not being closed because the site was wanted for an alternative use—either for aircraft noise testing or for some defence use, although the possibility of using the

site to study engine noise was being considered.

Mr Neave also revealed at the hearing that the government had refused to make available the minutes of three meetings held between Hawker Siddeley and the government during the course of last year to discuss the future of Tracked Hovercraft in general and of linear motors in particular. The committee want to see the minutes partly because there are not inconsiderable discrepancies between what Hawker Siddeley say happened at those meetings and what the government consider took place.

SCIENCE POLICY

Aigrain for MIT

PROFESSOR PIERRE AIGRAIN, science adviser to the French government, is to leave his job as Délégué Général for scientific research and technology at the Ministry for Industrial and Scientific Development. He will be taking a one year visiting professorship at the Massachusetts Institute of Technology.

Professor Aigrain's decision to leave the French government's service comes as no particular surprise as it is now five

What is rather more surprising is that Professor Aigrain plans to teach for a year rather than remain in the field of science policy.

The European Commission has still to appoint a science adviser, and Pierre Aigrain was heavily tipped as the man for the job. Since 1968 Professor Aigrain has been chairman of PREST—the European Commission's working group on scientific and technical research policy—and he is also chairman of the more recently formed COST group, a similar organization with a membership of nineteen European nations. As such he has been one of the most influential voices in research and development matters in Europe, and has been closely in touch with the commission's thinking on research and development.

Since the reshuffle of commissioners' jobs with the entry of Britain, Denmark and Ireland to the community on January 1 this year, however, it has not been certain that the commission will in fact appoint a full-time science adviser. The idea of such an appointment was Mr Altiero Spinelli's, but now Mr Ralf Dahrendorf has responsibility for such matters and he has not decided whether the appointment should be made.

Professor Aigrain will no longer be part of COST and PREST as he will no longer be the French delegate, and it is out of the question that he could become the commission's science adviser while he is at MIT, where he intends to teach solid state physics. But the appointment is only for a year, and his plans after that are not known.

Professor Aigrain's successor at the Délégation Générale has yet to be appointed although an announcement is expected late this month or early next. Dr Hubert Curien, Director General of the Centre National de la Recherche Scientifique is thought to be the likeliest candidate.



—ORTF, J. C. Mallinjad

Professor Pierre Aigrain

years since he took up the appointment of science adviser. The Délégation Générale à la Recherche Scientifique et Technique was created in 1958, and all three of its heads have now left after holding the post for five years although there is no stipulated term of office.

NEW WORLD

Infant Care takes a Hammering

by our Washington Correspondent

To the embarrassment of some and the concern of many, the United States, for all its material wealth, has a high rate of infant mortality. The rate is, in fact, higher than in some 13 other countries. That statistic has, not surprisingly, been widely used as a stick with which to beat the health care system in the United States, particularly for failing to provide adequate services to the poor and to minority groups. Last week, such charges were placed on a much firmer footing with the publication of a meticulous study of birth records in New York City (*Infant Death: An Analysis by Maternal Risk and Health Care*, available from the National Academy of Sciences, 2101 Constitution Ave., Washington DC 20418. \$6.00 paper, \$8.00 cloth).

Carried out under the auspices of the Institute of Medicine, part of the National Academy of Sciences, the study involved examining the records of all the births which took place in New York in 1968—more than 140,000 in all. It was found that 21.9 infants in every 1,000 born that year died before they were 12 months old. Their chances of survival were found to be strongly correlated with the quality of health care that their mothers received during pregnancy, and the group that carried out the study found that there is a "gross misallocation of services by ethnic group". If all the women in New York who gave birth in 1968 had received adequate prenatal care, infant mortality could have been cut by one third, the study suggests.

A central feature of the study was an attempt to determine, on the basis of social and medical factors, which mothers run a risk of having infants with lower chances of survival, and then to see whether the risks are ameliorated by proper health care during pregnancy. Social risk was determined on the basis of such factors as the mother's education, age, marital status and number of children, while medical risk took into account such factors as blood pressure, pelvic size and so on. All such data were contained in the 1968 New York birth records.

The correlations between high risk groups and infant mortality are striking. Those infants whose mothers were assigned to both the social and medical risk groups had a mortality rate of 42 per thousand in the first year of their lives; those whose mothers were

assigned to either the medical or the social risk group had mortality rates of 27.7 per 1,000 and 24.9 per 1,000 respectively, and those whose mothers came from the no-risk group had a mortality rate as low as 12.1 per thousand.

As for the effect of health care, the study rated the care according to the number of visits the mother made to her doctor during pregnancy, the time of her first visit and the kind of hospital service during delivery. Again, the correlation is striking with, on average, a two-fold difference in mortality rates from 13.3 per 1,000 infants of mothers deemed to have had adequate health care, to 35.6 per 1,000 infants whose mothers received inadequate care.

But those figures tell only half the story, for there is a very marked racial imbalance not only among the risk groups, but also according to the quality of health care received.

Some 60 per cent of the white, native born mothers were placed in the no-risk group, compared with only 25 per cent of the black mothers. Moreover, about three times as many black women as white were assigned to the social risk group. As for quality of health care, some 43.3 per cent of the white, native-born mothers received adequate care

during pregnancy, while only 4.6 per cent of black, native-born women were placed in that category.

An important aspect of the study is that it is easy to identify those women in the high risk groups on the basis of a few simple questions asked during their first visit to the doctor after they become pregnant. The study thus suggests that the type of health care should be based on the risk categories, and recommends that the American College of Obstetricians and Gynaecologists should draw up appropriate guidelines for physicians.

But the problem is that health care seems to be so grossly misallocated at present that it will require a radical change in the whole health care system to rectify. According to the study, some 70 per cent of the women who received inadequate health care were also deemed to be at either social or medical risk or both. Even worse, among the 22,000 black and Puerto Rican mothers in the social risk group, less than 2 per cent received adequate health care.

Small wonder, therefore, that Dr Robert Coles of Harvard University said in a preface to the report that "we do things wrong, we are indifferent to the needs of others—and here, right here is the proof."

TECHNOLOGY ASSESSMENT

Setback for OTA

by our Washington Correspondent

THE Office of Technology Assessment (OTA), Congress's new advisory and investigative body for technological matters, has received yet another setback. For the past nine months, the OTA has existed in name only and, thanks to a little-noticed provision that came as part of Congress's historic ban on the bombing of Cambodia, it is going to remain that way for some time. The root of the problem is the tortuous appropriations process.

In short, although the OTA was established by a bill passed in October last year, it cannot get under way, no director can be appointed and no staff taken on until it gets some money. And that requires a separate act of Congress. Supporters of the OTA, led by Senator Edward M. Kennedy, who has been elected chairman of the office's 12-member policy board, decided that the quickest way to get funds would be to tack an amendment to the Supplemental Appropriations Bill when it came up for

a vote in the Senate in May. (The Supplemental Appropriations Bill provided additional funds for a sheaf of government agencies for the 1973 fiscal year.) But, unfortunately for the OTA, the Supplemental Appropriations Bill was turned into a controversial affair when an amendment cutting off funds for hostilities in Indochina was also attached to it. The version passed by the House of Representatives contained a much weaker antiwar provision and the whole issue got bottled up in a conference committee for weeks.

When the bill finally emerged from conference at the end of June, the funding for OTA had been dropped, reportedly because with only a few days of the fiscal year left to run, the house conferees argued that it was not worth providing money for a new agency. The money would, however, have allowed a director and staff to have been appointed, and some studies to be initiated. The upshot is that the OTA must wait until the appropriations for the legislative branch are passed by Congress, and that is unlikely to happen until the end of September at the very earliest.

AAAS

Science and Man in the Americas

The American Association for the Advancement of Science and the newly-formed Mexican National Council on Science and Technology (Conacyt) jointly sponsored a meeting in Mexico City from June 20 to July 4 on the theme Science and Man in the Americas. In this article our Washington Correspondent describes and discusses the meeting.

SOME 450 years ago, Hernando Cortes and his small army of Spanish invaders conquered and effectively destroyed this ancient Aztec city. During the past two weeks it has been invaded again, this time by an army of some 6,000 scientists bent not on destruction but on helping development. Their objective was to discuss ways in which science and technology can be brought effectively to bear on the staggering problems of the developing countries of Latin America. They will clearly be less effective in dealing with the problems than Cortes was in destroying the cities, but most of the organizers of this meeting believe they have made a good start.

Like any large international scientific gathering, the most productive meetings took place over coffee, in bars, or in the corridors rather than in the scheduled sessions—a fact which makes the event difficult to describe. Nevertheless, a few themes were common to all the sessions, the most important of which was a clear desire to stay away from overt political wrangling. It goes without saying, however, that virtually all aspects of development in Latin America are underpinned by political considerations, and the meeting was often at its most interesting when such considerations actually emerged at the surface.

There can scarcely be a more appropriate setting for a meeting on Science and Man in the Americas than Mexico City. One of the most elegant and lively cities in the Western Hemisphere, it also has most of the problems endemic to the developing countries, as anybody who ventures out of the rich sections of the inner city (or who drinks the water) will testify. It has also tasted the fruits of technology and run up against some of the problems of the cities in more developed countries—the air pollution, for example, is as bad as anywhere in North America. Most important, the city is experiencing a burgeoning growth in its population, exacerbated by an

exodus of people from the rural to the urban areas.

With those problems as a backdrop, the meeting kicked off with a three-day symposium on technology transfer, without doubt one of the most important and politically explosive topics to be dealt with at the conference. The export of technology from the rich nations, particularly from the United States, to the developing countries is the process by which most developing countries receive the lion's share of their technology. But, along with the technology, they also import a good many problems, both political and economic, and in recognition of that fact many Latin American governments have in the past few years adopted laws to control and guide technology transfer and to limit foreign investment. Yet, in the three days that were devoted to the topic, there was little concrete discussion of the way in which Latin American economies are shaped by the transfer of technology from the United States.

Multinational Corporations

Chief conduit for this one-way flow of technology from the rich nations into the poor are multinational corporations. They are also the objects of intense suspicion (not surprisingly, in view of the antics of ITT in Chile) and lately of close examination. Dr Robert Staubaugh of Harvard Business School told the symposium of his own unpublished studies of multinational corporations operating in Latin America. According to his figures, "it appears that US multinational enterprises account for approximately 90 per cent of the total US exports of technology for which the US receives fees and royalties" and they also account for more than half of the exports of manufactured goods from the US, he said.

As for research and development, less than 10 per cent of the efforts of US multinationals take place outside the US, and of that, less than 10 per cent is in

developing countries. That fact is perhaps good reason why many developing countries do not get the technology that best serves their needs. Moreover, Staubaugh's study turned up the finding that the management structure of subsidiaries of US multinational enterprises is heavily weighted in favour of US citizens—only about 20 per cent of the general managers of such subsidiaries are drawn from the host country.

Nevertheless, the burden of Staubaugh's message is that on balance, multinational corporations bring more benefits than disadvantages to developing countries, and that Latin American governments should negotiate with the enterprises to make sure that the disadvantages are minimized. And that is precisely what several of those governments are now trying to do.

Avoiding Bad Deals

In particular, Mexico recently passed two laws, one to regulate transfer of technology into the country in an attempt to make sure that the imported technology is suited to Mexico's needs, and the other to limit foreign investment, with the long-term goal of Mexicanizing industry. According to Dr Gerardo Bueno, the Director-General of Conacyt, the impetus for the new laws came from a study of five sectors of Mexico's economy—automobiles, primary and secondary pharmaceuticals, and primary and secondary chemicals. The study brought to light not only the fact that many Mexican companies were getting extremely bad deals from their American suppliers—overpricing of technical services, restrictive clauses preventing re-export of products and so on—but it also turned up some undesirable factors which underpin many operations of multinational corporations. Not the least important of such factors is that technical decisions tend to be taken in the corporation's headquarters, with the result that they do not necessarily take into account the needs of the countries in which they are operating.

The first of Mexico's two new laws, which came into effect at the end of last year, sets up a central registry for all items of technology transfer into Mexico, and it gives the Ministry of Industry and Commerce the power to prevent those transfers which are considered to be against Mexico's interests. For example, the ministry will not allow Mexican companies to import technology which can be obtained in Mexico,

and it will veto contracts which place restrictions on exports and so on. Whether the law will enable Mexico to attract the type of technology it wants and to keep out others is a moot point, but Dr Bueno said in an interview that he believes its chief effect will be to give legal backing to Mexican companies in their negotiations with US enterprises.

Potentially more important is the new law which limits foreign investment in Mexico. As a general rule, all corporations established in the country from now on must have at least majority Mexican control, but there is some confusion about how the law will be interpreted. For one thing the committee of ministers which is supposed to oversee its workings has yet to meet, and for another, it is not at all clear how the law will affect enterprises already established in Mexico. The Mexican constitution forbids laws to be made retroactive, but one interpretation of the investment law is that foreign companies now operating in Mexico will not be able to expand their enterprises unless they comply.

In any case, those officials concerned with administering the new laws are well aware that in attempting to get the investment and the technologies which best serve Mexico's needs, they run the danger of scaring off other potential investors. Counsellors in some of the foreign embassies in Mexico City in fact report that the investment law has already had such an effect, although it only formally came into force in May.

But, considering the burgeoning population growth in Latin America, and the extremely high unemployment rates in virtually every city in the continent, does Latin America really want to import capital intensive industries? Will that not, in fact, exacerbate the unemployment problem? Bueno agrees that capital intensive industries may not always be in Mexico's best interests, but he argues, however, that in some sectors such as steel and fertilizers, which have a large effect on other sectors, efficiency is the primary requisite. The trick, of course, is to try to attract investment chiefly in those areas.

Population and Malnutrition

Nevertheless, it is perhaps a truism to say that the most pressing problems in Latin America are population growth and malnutrition, both of which were the subjects of central themes of the conference. Latin America has the fastest growing population in the world—it is growing at 3 per cent a year so that by the year 2,000, the present population of 270 million will number 600 million. According to Sol Linowitz, former Ambassador to the Organization of American States and now Chairman of the League of Cities, if present trends continue, by the end of the

century Latin America will be twice as populous as the United States and ten times poorer. Grafted onto this staggering growth rate is also a pronounced drift from the rural areas into the cities, a factor which greatly increases unemployment and other urban problems.

In spite of the implications of unfettered population growth for the future of Latin America, however, a three-day symposium on the topic turned out to be disappointingly academic and largely irrelevant. In fact, had it not been for an interjection by Dr Mario Margulis, of the Universidad de La Plata, Argentina, in the closing session, the symposium would have accomplished the remarkable feat of ignoring most of the contentious issues. As it was, the influence of the Catholic Church on population control in Latin America was not discussed.

What Margulis did was to question the motives of US agencies, foundations and corporations prominent in providing aid for population control in Latin America. He suggested that many of those corporations have been reaping large profits from Latin America, and that they are "partially responsible for the poverty" there. Their motives, he said, are chiefly to try to head off the political repercussions that stem from high population growth and increasing unemployment, and that humanitarian considerations scarcely enter the picture. Not surprisingly, that suggestion was not taken lying down by some of the other participants. But to judge by the applause, Margulis was not without his supporters.

As it turned out, a symposium on nutrition and human development provided as good a basis as any for discussing population policies, but in line with the rest of the meeting, most of the discussion of public policy issues took place not in the symposium but in chats afterwards. In particular, Dr Aaron Lechtig, of the Instituto de Nutricion de Centroamerica y Panama (INCAP), presented the results of a study of the effects of moderate malnutrition on placenta size, birthweight and infant survival. His studies show that a pronounced effect of malnutrition among pregnant women is a decrease in placenta size, and that this effect can be mitigated by improving the nutrition of the mother during pregnancy. Low placenta weight, he found, is closely correlated with low birthweight and high rates of infant mortality.

In an interview, Lechtig said that in his experience in rural villages in Eastern Guatemala, one of the chief reasons why families have so many children is that they know that many of them will die in their early years of life. He thus suggested that improvements in maternal nutrition will decrease infant mortality which will, in turn, lower fertility rates. Dr Roy E. Brown, of Mount Sinai

School of Medicine agreed. In one of the more relevant papers delivered at the population symposium, Brown argued that "we can expect a fall in fertility within a few years of a visible decline in (infant) mortality". Lechtig also criticized the approach of the US Agency for International Development, the chief supporter of family planning services in Latin America, for simply dispensing contraceptives without attempting to improve other social services.

Success or Failure?

Was the meeting, overall, a success? It is impossible to use any objective criteria to judge such an event, but at least the organizers were thoroughly pleased with the way it turned out. Dr Glenn Seaborg, Chairman of the board of the AAAS, said that he thought the meeting went "better than expected", a sentiment that was echoed by Bueno. Nevertheless, it is easy to fault some aspects. Canada, for example, was underrepresented—for one thing, it has been on the receiving end of perhaps more US technology exports than any other country, yet there was nobody from Canada to speak at the symposium on technology transfer. And if Canada was underrepresented, Cuba was ignored entirely. But perhaps the chief criticism is that in staying away from political issues as far as possible, too much of the meeting failed to get to grips with the most pressing issues facing Latin America.

A more radical critique of the meeting was provided by a small group from the organization SESPA, Science for the People, but the dozen or so members of the organization who got to Mexico City adopted a much lower profile at this meeting than they have at the previous few AAAS gatherings. They set up a literature stall outside the main lecture rooms, chiefly to put forward their view that the underlying rationale for the meeting was to aid US corporations and the US government in their exploitation of Latin America. Apart from a few minor arguments with the meeting's organizers, and one incident in which six SESPA members were detained by the police for 5 hours for distributing leaflets outside the building, their presence was little noticed.

In the long run, the chief effect of the meeting is likely to be the contacts that were fostered between US scientists and their counterparts in Latin America. And if Seaborg gets his way, those contacts could become cemented into a new organization for the advancement of science encompassing the whole of the Americas. That suggestion, at least, was put forward by Seaborg in his address to the meeting, and he proposed that such gatherings should be held on a regular basis—perhaps every three or five years.

NEWS AND VIEWS

Formation of Earth Craters

WHEN considering the plethora of lunar craters it is always enlightening to realize that the surface of the Earth has been cratered in a very similar way. It is, however, an indication of the strength of erosive processes that very few of the craters on Earth remain visible today. There are about sixty proven terrestrial impact sites, a number which compares rather unfavourably with the 300,000 craters visible on the near side of the Moon.

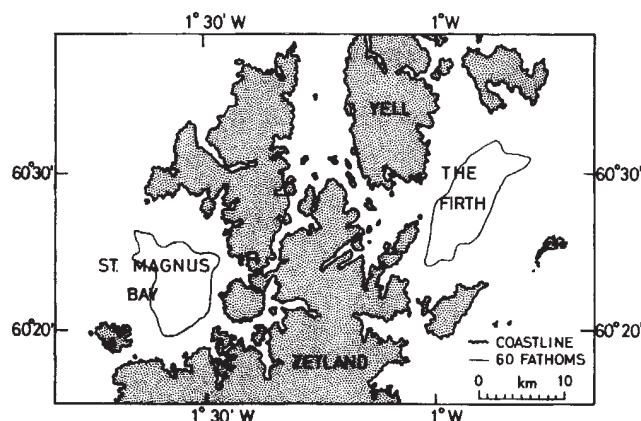
How do craters form? The Solar System, apart from containing planets and their satellites, also contains meteorites and asteroids. As an example, a large meteorite might have a mass of about 1 million tons and be moving with a geocentric velocity of 30 km s^{-1} . This body has a kinetic energy of about 10^{25} erg and the Earth's atmosphere would cause negligible retardation, the incoming meteorite appearing just as a brilliant fireball. This meteorite would crash against the Earth's surface at full speed, penetrate into the crust like a bullet and bury itself well underneath the surface before coming to rest. The kinetic energy would be converted into heat, sufficient to volatilize completely the whole impinging mass and convert it into an extremely hot gas bubble of temperature of the order of $1,000,000^\circ \text{C}$ imprisoned some two diameters of the original body beneath the Earth's surface. This gas bubble cannot be contained by the overlying debris and immediately explodes, like a point charge, producing a large crater. Very little of the original meteorite remains. A kinetic energy of 10^{20} erg would produce a crater of diameter 1 km, 10^{30} erg a crater of around 75 km. A perfect example of a meteorite crater is the 1.2 km diameter Barringer Crater in Arizona which was formed 30,000 yr ago.

Geophysicists and geologists have at their disposal

many criteria for recognizing a possible terrestrial impact feature. These features generally have a circular form with a central depression and a raised rim, the rim containing radial fracturing. The ones which have not suffered erosion contain traces of meteoritic material, usually in the form of iron shale and nickel-iron spherules. Impact produces surface breccia and sub-surface shatter cones and also small quantities of igneous materials—rocks, lavas and glasses—formed by impact melting.

Two probable meteorite craters have been found in Britain by Flinn (Department of Geology, University of Liverpool). At present these appear as two submarine deeps known as St Magnus Bay and The Firth, which are situated in the Shetlands, a group of islands in the northern North Sea about 320 km west of Bergen and 160 km north of Scotland. Both bays are around 90 fathoms (165 m) deep in the centre—a quite unprecedented depth for coastal waters in this region. It can be seen from the figure that the St Magnus Bay and The Firth craters originally had diameters of about 11 and 14 km respectively and both craters are now oval in shape, most likely because of local rock compression in an east-west direction. Flinn (*Proc. geol. Soc. Lond.*, No. 1663, 131; 1970) concludes that the two craters were probably formed in late Tertiary times, that is, before the Ice Age. Ice erosion, which was severe in the Shetlands region, has removed parts of the crater rim and has also "cleaned out" the craters. Their close spatial proximity suggests that these two craters were formed at the same time by two pieces of a disintegrating large meteorite. Craters of the size of St Magnus Bay and The Firth would be formed by impacting meteorites of masses about 1 million tons. The impinging bodies would be about 40 m across. The tendency for large meteorites to disintegrate and fall as showers is well known and there are quite a few examples of multiple craters, the two Clearwater Lake craters in Quebec, Canada, being one and the Ries Kessel and Steinheim structure of West Germany being another. These latter craters are separated by some 50 km, compared with the 36 km between the two in Shetland.

One of the interesting problems in this field is the determination of the terrestrial rate of impact cratering. Craters have been found with ages up to 570 million years, these early Phanerozoic craters being preserved on a Precambrian surface. Evidence for variations in the rate of infall of meteorites is somewhat inconclusive, for the situation is exacerbated by the differing degrees of severity of the various glacial erosion processes. This problem has been re-examined recently by Dent in the *Bulletin of the Geological Society of America* (84, 1667; 1973). He considers the spatial and temporal distribu-



Two probable submarine meteorite craters in the Shetland Islands; St Magnus Bay and The Firth have diameters of 11 and 14 km respectively and are 36 km apart.

tion of impact craters on the Canadian Shield and the effect on these craters of glacial exhumation. Two possible theories have been put forward to explain the observations. The classical one is due to Baldwin (*Icarus*, **14**, 36; 1971) and considers the Canadian Shield as a stable platform on which craters have steadily accumulated throughout Phanerozoic time. All craters of diameters greater than 10 km still remain, only smaller ones being lost by erosion. On the other hand, White (*Bull. Geol. Soc. Am.*, **83**, 1037; 1972) suggests that Pleistocene glaciation has eroded a significant depth of rock; before this the Shield was an area which accumulated flat-lying sediments much as the central part of the United States. White's theory explains why Palaeozoic and Mesozoic craters are found on a Precambrian surface and why Phanerozoic marine sedimentations are present in almost all the Canadian craters. The two theories also predict different influx rates. The distribution by age of Phanerozoic craters shows a peak 350 m.y. ago.

Now lunar investigations show that lunar cratering has been declining steadily for the past 4,000 m.y. with a half life of 250 m.y. If the cratering rate on the Canadian Shield was similar, early Phanerozoic craters would be four times more common than those formed in the past 100 m.y. Continuous erosion obviously has the opposite effect, making older craters harder to find. With erosion one would expect the crater age distribution to peak in recent times. In fact the opposite is true and there are considerably more old craters (ages between 250 m.y. and 500 m.y.) than new ones (ages less than 250 m.y.). Thus Baldwin's theory, which postulated a variable influx rate peaking some 350 m.y. ago, needs modification and Dent concludes that the age distribution of Canadian craters can be explained with a geological history of Canada based on White's glaciation theory and that no variation in the influx rate need be evoked. So the influx rate to the Earth is slightly dissimilar to that to the Moon. Tentatively the Earth influx rate can be put at such a value that one crater of diameter greater than 1 km forms every 50,000 yr. D. W. H.

Blowing Bubbles

THE recent discovery that clusters of galaxies also contain a detectable amount of hot gas in which the individual galaxies are immersed calls for a re-examination of radio galaxy models. Such a re-examination has now been carried out by Gull and Northover of the Mullard Radio Astronomy Observatory at Cambridge and their analysis is reported on page 80 of this issue of *Nature*.

Until recently, little thought was given to the existence of material in the space between galaxies—the intergalactic medium as it is called. It has been generally accepted that intergalactic space is the closest thing to a vacuum that one is likely to come across in nature. The fact that galaxies occur in clusters has modified that view only slightly—the space between galaxies in a cluster has generally been assumed to be a little less like a vacuum than the space between clusters, but in both cases only an upper limit can be given to the amount of material present.

Like much else in astronomy that concept was changed with the launch of the first X-ray satellite in 1970. The Uhuru satellite detected enough X-ray emission from clusters of galaxies to indicate the existence in these clusters of clouds of hot gas. Where this gas comes from is not immediately clear, but obviously its existence must be taken into account in considerations of the energetics of clusters of galaxies. This step has now been taken by Gull and Northover, and their view may well be the model with which observations of galaxies and quasars during the next few years will be compared.

Gull and Northover are, of course, particularly concerned with the appearance of galaxies in the radio telescope. The characteristic phenomenon here is the structure of the radio galaxies—frequently they consist of a pair of radio sources bracketing the optical galaxy. This dumb-bell shape is assumed to have been formed as a result of repeated explosions in the galactic nucleus.

What Gull and Northover have done is to examine what the effect of the intergalactic medium would be on the material thrown out in these galactic explosions. It turns out that the pair of radio sources can be interpreted as bubbles of material moving out into the intergalactic medium from their point of origin in the galaxy. In other words, Gull and Northover start with the proposition that bubbles of very hot plasma are generated in the nuclei of these galaxies by some mechanism which at present remains unknown. The crucial point then is that as this gas is less dense than the intergalactic medium, the bubble will rise, as it were, through the medium like a bubble of gas in water.

Much can be done by considering the balance between the internal pressure in the bubble and the external gas pressure, and by examining the theory of the way in which bubbles rise under gravity in a fluid. Gull and Northover have done these calculations, and come to the conclusion that there is no problem in containing even the most intense radio sources. By involving the intergalactic medium their description also allows an explanation of why there should be two and only two radio sources situated on either side of the nucleus.

This ticklish question is tackled from a consideration of the motion of the bubbles once they have left the nucleus. It seems that each bubble will develop into a nearly spherical cap followed by a highly turbulent wake. This wake is likely to disrupt any bubbles following immediately behind the initial two bubbles. And as Gull and Northover explain, there are reasons for supposing that the plasma released by a series of explosions in a galactic nucleus will not expand uniformly from the nucleus, but instead will expand preferentially along the rotation axis, forming an elongated cavity which eventually collapses in the middle into two separate bubbles.

This model is already reasonably successful in accounting for several features of radio galaxies—for example, the region of weak radio emission between the two chief sources is interpreted as originating from the wake, and Gull and Northover also predict various correlations between the size and power of the radio sources and the properties of the associated galaxy and galactic cluster. This aspect of their work will no doubt be the first to come under examination. But if this examination turns up no important snags it is likely that this model will be the yardstick against which radio observations of many extragalactic objects will be tested for the next year or so.

SKELETAL MUSCLE

Work-induced Growth

from a Correspondent

WORK-INDUCED skeletal muscle enlargement is common among athletes and physical culture enthusiasts. The mechanism by which this muscular enlargement takes place, however, is still not established, though some light is now shed on the subject by Jablecki, Heuser and Kaufman (*J. Cell. Biol.*, **57**, 743; 1973) who studied RNA synthesis in hypertrophying muscle of rats.

These authors confirm earlier observations (reviewed by Goldberg in *Cardiac Hypertrophy* (edit. by Alpert), Academic Press, 1971) that RNA synthesis is an important prerequisite to work-induced muscular growth and give evidence that the site for this RNA synthesis is connective tissue. They observed that treatment of animals with actinomycin (an inhibitor of RNA synthesis) prevented muscular hypertrophy and thus confirm that new RNA synthesis is essential. Localization of RNA synthesis was determined by autoradiography; comparisons of light autoradiographs of control and hypertrophying muscles injected with tritiated uridine showed there to be an increased deposit of silver grains in the hypertrophied muscle. The grains were deposited almost exclusively in the connective tissue, few grains being visible on the muscle fibres themselves. Evidence that the radioactivity was associated with RNA was given by the absence of silver deposit on the autoradiograph when the animal had been pretreated with actinomycin D.

During hypertrophy there was a concomitant increase in proliferation of connective tissue, as had been observed by Sobel and Kaufman (*Archs Biochem. Biophys.*, **137**, 469; 1970) who noticed that in the distal portion of the muscle proliferation was most extensive; they therefore used the central "less contaminated" portion of the muscle for their studies and hence failed to associate RNA synthesis with connective tissue. Jablecki *et al.* found a dramatic difference when comparing the rates of uptake of tritiated uridine in the distal and proximal portions of hypertrophying and control muscles: in hypertrophied muscle, the distal portion incorporated 320% more radioactivity into RNA than the control as compared with a 60% increase above the control level in the proximal portion. Conversely, preliminary experiments suggest that decreased activity of muscles, produced by spinal section, may be accompanied by a decreased rate of RNA synthesis; however, further experiments are necessary to verify this.

The work of Jablecki *et al.* can be correlated with observations on hyper-

trophying cardiac muscle: there is a transient increase in rate of RNA synthesis during hypertrophy; hypertrophy is also accompanied by proliferation of connective tissue. The site of RNA synthesis, however, remains to be determined. Although skeletal and heart muscles are known to be very different new ideas pertaining to the mechanism for hypertrophy in skeletal muscle should stimulate similar investigations on cardiac muscle.

ECHOLOCATION

Tuning in to a Target

from our Animal Behaviour Correspondent

THE horseshoe bats (*Rhinolophidae*) echolocate by emitting an ultrasonic cry through the nose and beaming it by means of the nose "leaf". The cry consists of a long part of constant frequency, followed by a short frequency-modulated pulse. It seems likely that the time lapse between this f.m. terminal flourish and the f.m. part of the returning echo gives the bat information about the distance between it and its target. The long constant frequency portion, on the other hand, seems to be used to measure the relative speed of the bat and the target. As the bat flies towards a target, the echo from the cry will be higher in frequency than the cry itself because of the Doppler shift.

For a stationary target, the extent of the Doppler increase will be a function of the bat's own flight speed. It has been shown that the bat's auditory system is especially sensitive to the frequency of the bat's own cry Doppler-shifted by this amount. Neurophysiological studies of the inferior colliculus

of *Rhinolophus* have shown that this auditory part of the brain responds both when the echo begins ("on-response") and when it ends ("off-response"). The maximum on-response is obtained at a frequency of 0.5 kHz above that of the off-response. H.-U. Schnitzler (*J. comp. Physiol.*, **82**, 79; 1973) now shows that *Rhinolophus* has a very sensitive feedback system which acts to keep the frequency of the emitted cries such that the returning echo is just at the best frequency for the off-response, so giving the bat high sensitivity to its own echo. He experimentally altered the amount of Doppler shift which a bat's cry underwent by making the animal fly in a He-O₂ mixture, where the speed of sound is higher than in air. The bat correctly compensated for this change, and altered the frequency of its outgoing cries so that the echoes still returned at the best frequency for the off-response.

Schnitzler suggests that by having the on-response of the evoked potentials in the inferior colliculus tuned to a slightly higher frequency than that of the off-responses, the bat can be very sensitive to unexpected movement of the target itself. If an object moves towards a bat, this will impose an additional Doppler increase on the echo, which will then fall into the frequency area of the lowest on-threshold.

This finely tuned feedback system thus not only enables the bat to hear its own echoes particularly sharply when the target is not moving (using the off-responses), but it also facilitates the detection of additional target movement (using the on-responses). How the system works with such accuracy is far from understood. The problems of such things as how the bat compares the

More About Foetal Haemoglobin

THE discovery by Schroeder and Huisman and their coworkers in 1968 that the γ chain of human foetal haemoglobin (Hb-F) is heterogeneous, and that the two types of γ chain— $G\gamma$ and $A\gamma$, with a glycyl or an alanil residue at position 136 respectively—are the products of non-allelic structural genes, has made possible the study of several new features of the developmental control mechanisms for globin chain synthesis.

In 1971, the same group of workers showed that the 70:30 ratio of $G\gamma$ to $A\gamma$ chains found at birth decreases during the first year of life until the adult ratio of 40:60 is attained. Thus the repression of γ -chain synthesis and activation of β - and δ -chain synthesis coincides with a change in the relative activities of the $G\gamma$ and $A\gamma$ genes. More recently, in 1972, a study of Hb-F variants abnormal in either the $G\gamma$ or $A\gamma$ chain led them to the conclusion that there are in fact four non-allelic γ -chain structural gene loci ($G_m\gamma$, $G_z\gamma$, $A_m\gamma$

and $A_z\gamma$) with relative activities at birth of approximately 4:2:2:1. The proportions of the variants at birth indicated that Hb-F (Malta I) and Hb-F_x (Negro) were produced by mutants at the $G_m\gamma$ and $G_z\gamma$ loci respectively.

Further support for this striking example of gene multiplicity has now come from a study (see Huisman in *Nature New Biology* for July 18) of the postnatal changes in the relative properties of these two variants in heterozygotes during the first six months of life. For Hb-F (Malta I) the average proportion (relative to total Hb-F) declined from about 37% at birth to about 13% at 72–134 days of age. For Hb-F_x (Negro), by contrast, the average proportion at birth (about 11%) showed no postnatal decrease. This difference is consistent with the view that there are indeed two $G\gamma$ structural loci, with separate control mechanisms operating during the $\gamma \rightarrow \beta$ switch.

echo with its criterion echo frequency or of how it manages to control its emission frequency of about 83 kHz to within ± 100 Hz, are formidable.

PROTEIN SYNTHESIS

Every Dogma has its Day

from our Molecular Biology Correspondent

WHATEVER displeasure the discovery of reverse transcriptase may have caused in exposing a rift in the Central Dogma, it now appears that, even aside from dialectical considerations, it may simply as a technical innovation come to dominate the next epoch of molecular biology. The observation that if fed poly(dT) it will make the complementary DNA from an RNA with a poly(A) tract at its end—that is to say practically any eukaryotic messenger that is offered to it—has opened a veritable cornucopia of new and exciting possibilities. One such devolves on the estimation of messenger concentrations, and two groups of workers have now perceived how this approach can be used to probe deeper into the origins of thalassaemia.

The thalassaemias are a group of syndromes of two general types— β thalassaemia, in which there is a dearth or even total absence of β chains, and foetal (γ) chains appear in their place in the finished haemoglobin, and α thalassaemia in which surplus β chains appear as haemoglobin H, that is to say, β_4 . It is known that the α - and β -chain messengers are associated with polysomes of appreciably different sizes, and give rise normally to exactly balanced quantities of the two globin chains. The haemoglobin messenger isolated from β -thalassaemia patients, on the other hand, when translated in an animal cell-free system, produces a large preponderance of α chains, and contrariwise in α thalassaemia. This should signify that one of the messengers is in short supply, but there remains a reservation, namely that the other messenger might be present but defective, in being unable to bind to the ribosomes, or for some other reason incapable of translation.

It is this doubt that has now been resolved. Housman *et al.* (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1809; 1973) have measured the concentrations of the two messengers directly by quantitative hybridization with their complementary DNAs, prepared by reverse transcription. For this purpose one needs, of course, reasonably pure samples of messengers to begin with, and these can be obtained by collecting the appropriate polysome fractions. A purity of some 90% was achieved, using rabbit reticulocyte material. The DNA prepared from the messenger template can then be titrated with the messenger to establish the equivalent point. Housman *et al.* have gone to some pains to

show that the DNA is indeed the unique complementary product: this was demonstrated by nucleotide mapping, and by the absence of significant cross-hybridization between the RNA for one globin chain and the DNA for the other. With these preparations the assays for the α - and β -chain messengers give values respectively of 8:1 and 6:1 for the ratio of the right to the wrong species.

The evolutionary conservation of the sequences is sufficient to allow the rabbit DNA to be used for the assay of human messengers. The degree of hybridization to be sure is less, but correction can be made in terms of a calibration. With messenger isolated from α -thalassaemia blood then, a six-fold excess of β over α chain was found, whereas in β thalassaemia the ratio was 10:1 in favour of the α chain. In the latter case, however, it is known that there is abundant γ -chain synthesis, and therefore in order to eliminate any ambiguity resulting from attachment of γ messenger in place of the not too dissimilar β species human β -chain DNA was synthesized to give improved complementarity. A highly enriched β -messenger preparation was isolated from α -thalassaemia cells for this purpose. Moreover, to achieve maximum discrimination from γ -chain RNA, the conditions were made more stringent by increasing the hybridization temperature. This led to an α/β ratio of 10:1.

Much the same results emerge from a similar study by Kacian *et al.* (*ibid.*, 1886), which, however, cuts a corner by using a mixture of α - and β -chain DNAs, one labelled with ^{32}P , the other with tritium. In normal cells, or those from patients with haemolytic conditions other than thalassaemia, the $\alpha:\beta$ mes-

senger ratio proved to be close to unity, whereas for α and β thalassaemia the balance favoured β - and α -chain messengers respectively. The ratios were less striking than those obtained by Housman *et al.*, who used a more exacting procedure, and Kacian *et al.* have also not attempted to come to terms with the problem of γ -chain messenger competition. The conclusions are in no doubt, however, and the next step presumably will be to find out why one or other messengers should be so scarce in these syndromes—whether for instance it is the mechanism of transport into the cytoplasm that is defunct, or the breakdown is at the transcriptional level.

As to the problem of how genes are activated, Barker *et al.* (*ibid.*, 1739) have evolved a promising system, in which haemoglobin synthesis can be switched in response to a hormone, erythropoietin. Goats, when young, manufacture a foetal haemoglobin, $\alpha_2\gamma_2$. This is then replaced by haemoglobin C ($\alpha_2\beta_2^C$), and in due course, in the appropriate genetic stock at least, by the adult form, haemoglobin A, or $\alpha_2\beta_2^A$.

The goat responds to induced anaemia by reverting to haemoglobin C synthesis and the same effect can be elicited in healthy animals by erythropoietin. Barker *et al.* have now shown that the system can be worked in tissue culture of bone-marrow cells with a change from A-chain to C-chain synthesis in the space of about three days. The evidence on whether the change involves the growth of new clones of cells or occurs by way of an intercellular mechanism is as yet equivocal. Rather similar experiments have also recently been carried out by Adamson and Stamatoyannopoulos (*Science*, **180**, 310; 1973) on sheep bone-marrow cells.

Make and Mend DNA

SPONTANEOUS depurination of DNA occurs at such a rapid rate (10^{-7} h^{-1}) that long term survival of genetic information would be unlikely were it not for the existence of a repair mechanism. An enzyme (still nameless) which may play a vital part in repairing depurinated DNA will be described by Verly and Paquette in *Nature New Biology* next Wednesday (July 18).

Verly and Paquette have purified the enzyme in question from both *Escherichia coli* and rat liver, and there is evidence of its occurrence in calf thymus and a plant as well.

The authors demonstrate that the enzyme makes single-stranded breaks in native DNA at apurinic sites. A highly sensitive test shows that native DNA is not attacked: after incubation with the purified enzyme the sedimentation rate of T7 DNA (after strand separation) is unaltered. Neither does the enzyme

digest single stranded DNA, but DNA methylated with methyl methanesulphonate is degraded at a rate which is consistent with the known tendency for alkylated purines to be eliminated spontaneously from DNA. DNA depurinated by heating is a good substrate for the enzyme. The authors know that the enzyme breaks the polydeoxyribonucleotide chain specifically at the sites of apurinic residues because they have shown that alkali treatment (which does the same thing) does not degrade the DNA further.

Verly and Paquette propose that when depurination of DNA occurs *in vivo* their enzyme makes single stranded scissions in the DNA next to the apurinic residue; DNA polymerase removes and repairs the damaged segment; and finally a ligase stitches the newly synthesized segment back into the neighbouring native sequence.

RODENTS

Guinea-pig Relatives

from a Correspondent

THE thirty-fourth Symposium of the Zoological Society of London, held on June 7 and 8, dealt with the biology of hystricomorph rodents. The guinea-pig is the best known hystricomorph but the delightful display of large photographs of some of its near and distant relatives was indicative of the variety of species represented in the programme. The meeting was organized by Drs I. W. Rowlands and B. J. Weir, of the Wellcome Institute of Comparative Physiology, which is part of the "zoological complex" in Regent's Park. Much of the work at the institute concerns the reproductive physiology of South American hystricomorph rodents and the breeding of about ten species has been for some years an outstanding feature of the research of this small but active unit.

The classification and phylogeny of the suborder Hystricomorpha are somewhat controversial. Taxonomically, the group is defined and classified according to a few anatomical features, chiefly those related to the insertion of the jaw muscles. The meeting began with a battle of taxonomic giants—Professor R. Lavocat (Université des Sciences et Techniques, Montpellier) and Professor A. E. Wood (Amherst College, Massachusetts) on the nature and origins of hystricomorphs under the chairmanship of Professor G. G. Simpson (Tucson, Arizona). Succeeding speakers all attempted to relate their findings to the taxonomy of the group, although they dealt with such widely differing aspects as cephalic arteries and chromosomes, patterns of behaviour and audiograms of animal noises, the ecology and the economic value of the African grass-cutter (a coypu-like creature prevalent in Ghana), reproductive characteristics, embryology and placentation, progesterone synthesis and metabolism, and the structure of hystricomorph insulins.

It became clear that these rodents share a characteristic placental structure and that they seem to maintain a high level of circulating progesterone, necessary for pregnancy, by a method which is peculiar to them. They produce unusual and variable insulins, they possess complex ovaries and are renowned for their slow foetal growth and long gestation periods. One species, the plains viscacha (*Lagostomus maximus*), has a bizarre reproductive habit in that it ovulates between 200 and 800 eggs from ovaries that look more like those of a fish than a mammal. Only about six or eight eggs are implanted after a short period of delay and twins are invariably delivered after a gestation of 5½ months; the excess embryos are

resorbed at a predictable time according to their order of implantation.

Little is known of hystricomorph ecology, but some films showing the diverse habitats of some of the animals indicated the importance of an awareness of their natural habits. Such knowledge has undoubtedly contributed to the success with which hystricomorph rodents have been bred at the Wellcome Institute.

GRAVITATIONAL RADIATION

The Ten Year Test

from a Correspondent

PROFESSOR J. WEBER is now detecting what may be gravitational waves at a rate of seven events per day. This was announced by Weber (University of Maryland) at a meeting "Ondes et Radiations Gravitationnelles", organized by CNRS, and held in Paris from June 18 to June 22. Although much of the programme was devoted to reports on the solution of relativistic field equations and on the interaction of gravitational waves with various media, interest centred on the discussion of new experimental work.

Weber's most recent data—obtained from about one month's running of the Argonne and Maryland cylindrical detectors in coincidence—have been analysed by a new and simpler computational method, and for the first time have been backed up by calibration tests with artificial pulses. He reports that the coincidences observed correspond to an increase in energy exceeding $kT/100$ in each detector ($>$ one hundredth of the mean thermal energy per normal mode in the detector). Weber has obtained time delay histograms showing a strong excess of coincident pulses over those expected by chance in experiments involving two simultaneous and separate methods of data recording. In one of these all the data were recorded at Maryland with a telephone line relaying the Argonne information and in the other the data were recorded separately at each site. The coincidences seem to be better defined in time than before, all being recorded within the central 0.1 s time interval in the distribution.

But other experimental groups had little in the way of positive results to present. Dr R. Drever reported the work of a group at the University of Glasgow which has been operating two split bar detectors placed 50 m apart in coincidence for about a year, these devices being arranged to be most sensitive to pulses of a few milliseconds duration or shorter. From seven months of analysed data a limit of 1 ± 3 coincident events per month has been set for short pulses which could deposit more than $kT/4$ energy in each detector; however, one very unusual coincident event has been recorded in which both detec-

tors were excited in exactly the manner expected for a short burst of gravitational radiation. Although the Glasgow detectors are smaller than those of Weber these experiments make it seem unlikely that the events which Weber was detecting a few years ago were gravitational pulses of millisecond duration.

There are now three detectors of similar design to that of Weber in Europe, one being operated by Dr S. Bonazzola at Meudon Observatory near Paris. The other two detectors, developed by Dr H. Billing at the Max Planck Institute, Munich, and by Dr K. Maischberger at Frascati, are operated in coincidence; Dr P. Kafka (Max Planck) reported that for 18 days running in April no coincidences were observed above chance at an excitation of more than $kT/4$ in the devices. Although this result was not claimed to be in conflict with Weber's present observations it was suggested that there might be some disagreement with his earlier results recorded with poorer sensitivity. But Weber replied that he could make no guarantee as to the constancy of the source. Kafka described a theoretical optimization procedure for both the Munich/Frascati and Weber's detectors and predicted a poorer sensitivity for the Argonne/Maryland devices than Weber has measured. In answer to this Weber disagreed that Kafka's ideas were an optimization and stated that when he tried such a procedure his sensitivity was poorer. It is to be hoped that the sensitivity question can be resolved soon because it is so fundamental to the interpretation of experiments.

The uncertainties in the experimental situation should be helped by a reported agreement by Weber to exchange magnetic data tapes with some of the experimental groups at the conference. This should allow a clearer comparison of the different experimental procedures as well as introducing the possibility of long baseline coincidence experiments.

Professor M. Rees (University of Sussex) recalled the theoretical difficulties in explaining a flux of gravitational waves at Weber's level (see also *Nature*, **243**, 61; 1973). Although considerations of energy loss from our Galaxy, together with beaming mechanisms, might just be stretched to accommodate these results he reaffirmed his views that in order to observe a few events per year from well known astrophysical processes a detector must be sensitive enough to detect supernovae in the Virgo cluster. This requires an improvement of about 10^8 over the present level of sensitivity. But he pointed out that an improvement of only 10^4 may enable the patient experimentalist to observe a pulse rate of at least one per 30 years

due to stellar collapses or supernovae in our Galaxy. Rees also considers that the detection by ground based techniques of theoretically predicted monochromatic gravitational radiation from pulsars or binary stars is more difficult than detection of pulses.

What does the future hold? Some experimental groups are already investigating techniques which may yield several orders of magnitude improvement in sensitivity over Weber's present level. For example, improvements in the range 10^6 to 10^8 were predicted by Dr M. Cerdonio (University of Rome) for the cryogenic detectors of 5 ton mass which are expected to be in operation at Stanford, Louisiana, and Rome in about 5 years. Further improvements will almost certainly be obtainable in space experiments. But as Professor K. Thorne (California Institute of Technology)—speaking on behalf of Dr R. Davies—mentioned, these tend to be limited by long lead times and high cost in the case of specialist experiments and the need for technological improvements if space missions planned for other purposes are to be used.

Hopefully gravitational radiation will be detected unequivocally within the next decade; as Thorne pointed out, when that happens the only significant experimental tests of some of the new metric theories of relativity may be possible. Most of these theories lead to distinctive polarization states for gravitational radiation and certain of them predict different propagation velocities for gravitational and electromagnetic waves, factors which should be amenable to experimental testing using either "Weber waves" or supernovae in the Virgo cluster.

EARTH'S MANTLE

Viscosity 10^{13} Poise ?

from our Geomagnetism Correspondent

ONE of the chief purposes of the precision levelling survey instituted in Iceland in 1966 is to measure the ground deformation associated with active volcanoes. The volcano Katla, which lies beneath the Myrdalsjökull ice field, is a case in point. During the past few hundred years there have been two important eruptions of Katla each century, the last being in 1918. On this crude statistical basis the volcano can be expected to break out again quite soon; and so to monitor its behaviour three arrays of levelling instruments were set up on the edge of Myrdalsjökull. Since 1966, however, Katla has shown no sign of activity, although the three arrays have nevertheless recorded significant tilt variations. What, then, is the cause of the tilt?

The results of the tilt measurements, which were recorded once a year at each

station during the period 1967–1971, have now been reported by Tryggvason (*J. geophys. Res.*, **78**, 2488; 1973). The amount of tilt between two measurements of level made about a year apart at the same array ranged from 1.4 to 4.5 μ rad. These values compare with an estimated probable error of about 0.5 μ rad in a year, and so cannot be attributed solely to effects such as the movement of bench marks. Moreover, from any one year to the next, the component of the tilt along the line joining an array to the centre of the ice field was always in the same direction for all three arrays—in other words,

over any given observational period, all three arrays were either tilted towards or away from the centre of the glacier. The possibility of this happening by chance, and thus the possibility that the tilts merely arise from random motions of the bench marks, must be very small or negligible. Curiously, however, the tilt direction alternated from one observational year to the next (towards the glacial centre one year, away from the centre the next), suggesting an oscillation with a period of about two years.

Tryggvason thus concludes that the tilt variations are significant and have a common physical origin. After reject-

No Magnetic Lineations over Cyprus

THERE is now a wide range of geophysical, geological and petrological data which suggests, or is at least consistent with the hypothesis, that the Troodos Massif of Cyprus is an upthrust fragment of Mesozoic ocean floor. To most, if not all, scientists familiar with the area the evidence seems to be overwhelmingly in favour of the idea; but it has to be admitted that the one piece of unequivocal evidence needed to clinch the case has yet to be obtained. Vine *et al.* clearly hoped that the vital data would emerge from a study of the magnetic anomalies over the area, for a body formed by seafloor spreading might be expected to produce the linear pattern characteristic of the present ocean floor. But in *Nature Physical Science* next week (July 16) they have to report that, once again, although the data are not inconsistent with an oceanic Troodos neither do they offer clear proof of an oceanic origin.

The basic data come from an aeromagnetic survey of southern Cyprus and adjacent offshore areas flown at a height of 2.6 km above mean sea level and comprising 5,000 km of continuous profiling. Unfortunately, the resulting anomaly map shows little sign of lineations. The largest anomalies and the steepest gradients are found to correspond with the Troodos igneous outcrop and particularly with the peripheral pillow lavas. But outside the outcropping regions the magnetic anomaly pattern correlates very well with the pattern of Bouguer anomalies previously reported by Gass and Masson-Smith (*Phil. Trans. R. Soc.*, **A255**, 417; 1963). This correlation, together with the fact that the pillow lavas correspond to positive magnetic anomalies, suggests that at least the pillow lavas are magnetized completely normally and that their magnetic anomalies arise from the surface and subsurface arrangements of the Massif—in short, that it is not necessary to invoke reversed magnetizations. This is consistent with the

palaeomagnetic study of Moores and Vine (*Phil. Trans. R. Soc.*, **A268**, 443; 1971) which failed to reveal any reversed samples from 150 sites within the Troodos complex.

The one suggestion of a hoped-for lineation lies to the east of the igneous outcrop and at the eastern end of the island where there is a pronounced north-south trending negative magnetic anomaly with a maximum amplitude of 500 γ . On the face of it, this is encouraging, for not only is this feature similar in form, amplitude and wavelength to the familiar oceanic linear anomalies, it is also parallel to the general strike direction of the Massif's dyke complex and thus perpendicular to the presumed spreading direction. But unfortunately it, too, corresponds to a similarly-shaped anomaly in the gravity field. Thus, although the area is known to be underlain by Troodos pillow lavas, it does not necessarily follow that the magnetic anomaly arises from a north-south strip of reversely magnetized material at depth. Other explanations are possible, involving normal magnetization and structural forms capable of accounting for both magnetic and gravity anomalies. In view of the gravity-magnetic anomaly coincidence these must be considered more likely than reversed magnetization.

The absence of reversed magnetizations is perhaps surprising, for a Massif formed by seafloor spreading at a rate of 1–2 cm yr⁻¹ would represent 10–15 $\times 10^9$ yr. Radiometric ages of Massif material, however, presented by Vine and his colleagues, confirm that the Massif is pre-Campanian (76 $\pm$ $\times 10^9$ yr). This means that the formation of the Massif probably coincided with the long Cretaceous Normal Polarity Interval first recognized by Helsley and Steiner (*Earth planet. Sci. Lett.*, **5**, 325; 1969) and recently shown by Irving and Couillard (*Nature phys. Sci.*, **244**, 10; 1973) to have lasted from 109 to 83 $\times 10^9$ yr ago. In short, the lack of reversals in Troodos material is sheer bad luck.

ing the swelling and shrinking of near-surface material (caused, for example, by variations in moisture content) as an improbable cause, he goes on to suggest that the tilt variations arise from changes in the load of the ice field. Variations in the total ice mass are known to be large because precipitation at higher elevations exceeds the equivalent of 400 cm of water a year and the climate in the region is relatively mild, but making an estimate of these variations is no easy matter. Nevertheless, by making various assumptions based on actual weather data and a few "arbitrary decisions", Tryggvason is able to demonstrate quite convincingly that variations in the ice budget correlate well with changes in the radial tilt component.

This may not be a particularly interesting conclusion in itself, but its consequences are altogether more significant in the light of the mechanism by which ice load is converted into tilt. Here there are apparently three possibilities; the variation in ice mass may change the direction of the gravitational pull, deform the crustal layers elastically, or deform the near-surface layers plastically. The change in the direction of gravitational pull may be calculated quite accurately if the distribution of the load is known; and by using appropriate data Tryggvason shows that the effect is to produce a tilt of only about $0.04 \mu\text{rad}$. In other words, this mechanism underestimates the observed tilt by a factor of about 100. Most of the observed tilt must therefore be the result of ground deformation.

Elastic deformation of the crust and mantle is difficult to estimate, but basing calculations on a model developed by Slichter and Caputo (*J. geophys. Res.*, **65**, 4151; 1960), Tryggvason is able to show that the tilt caused by elastic deformation is unlikely to exceed 10% of that observed. This, then, only leaves plastic deformation or liquid flow at depth. But the problem here is more complex in that the behaviour of the layers beneath Iceland is not well known. Thus it is not possible to proceed as in the other two cases by calculating a tilt which is then compared with the observed tilt. Instead, it is necessary to reverse the process by using the observed tilt to derive an appropriate Earth model. Tryggvason therefore attempts to determine the characteristics of a model in which an elastic plate overlies a fluid substratum and which is consistent with the tilt observations.

What this analysis shows is that the elastic plate, presumed to represent the lithosphere beneath the glacier, has a thickness of only 6.5–8.5 km. This is much thinner than the average oceanic lithosphere (about 70 km) but is not unexpected. Palmason (*Crustal Structure of Iceland from Explosion Seismology*, Reykjavik, 1970), for example,

concluded that the elastic crust beneath south-west Iceland is only 8 km thick (though rising to 15 km beneath north-west and south-east Iceland), and a melting point depth of about 10 km or less for basalt is a not unreasonable inference from heat flow data. As far as thickness is concerned, therefore, Tryggvason's result seems to be consistent with other evidence.

But viscosity is quite another story. The correlation between the estimated ice load and the observed tilt is apparently best when there is assumed to be little, if any, time lag between a change in load and the resulting tilt. In short, the response time of the system is close to zero. Feeding this information into a viscosity formula originally derived by Haskell (*Am. J. Sci.*, **33**, 22; 1937),

Tryggvason finds that the maximum viscosity of the fluid layer beneath the elastic plate is only approximately 10^{13} poise.

Most other estimates of upper mantle viscosity (generally calculated from the rate of uplift of deglaciated areas) lie in the range 10^{21} – 10^{23} poise, although Einarsson (*Jökull*, **16**, 157; 1966) estimated the viscosity below Iceland to be about 10^{20} poise. Thus Tryggvason's new viscosity is, remarkably, at least seven orders of magnitude lower than previous values. It is just possible, of course, that the value of 10^{13} poise refers not to any sub-lithospheric layer but to Katla's magma chamber. But this would make the chamber rather large, for the arrays lie about 15 km from the volcano.

***G. truncatulinoides* in Dispute**

A FEW months ago Theyer (*Nature phys. Sci.*, **241**, 142; 1973) presented palaeomagnetic and micropalaeontological data which seemed to invalidate the timing of what was thought to be one of palaeontology's most reliable datum planes—the first appearance of the planktonic foraminifer *Globorotalia truncatulinoides*. This plane had always been taken to mark the onset of the Pleistocene about 1.8 million years ago; and on this basis *G. truncatulinoides* would be expected to appear during the Matuyama reversed geomagnetic polarity epoch. The gist of Theyer's study, however, was that in the southern hemisphere south of 36° S, the foraminifer in question appears within the earlier Gauss normal epoch, or some 1.0–1.5 million years before its previously known appearance in lower latitudes. This is a serious matter for, clearly, if Theyer is right a great many studies based on the validity of this particular datum plane must surely have led to incorrect conclusions.

But Theyer is not to be allowed to get away with it, for in *Nature Physical Science* next Monday (July 16) he is attacked by Watkins *et al.* with a vehemence that is much less common in science than it was many decades ago. Thus Watkins and his colleagues claim that Theyer's conclusions are "totally wrong", that his palaeomagnetic work is "extraordinarily subjective" and fails to reach "even minimal acceptability standards", that some of the foraminifera illustrated by Theyer are misidentified, and that Theyer "has brought discredit to radiolarian biostratigraphy". These are hard words indeed, but they are also backed up by detailed criticisms. As far as the palaeomagnetic work is concerned, for example, Watkins *et al.* have examined not just Theyer's published article but also his unpublished thesis in which the methods are de-

scribed at some length. As a result, they find that Theyer has compiled polarity logs using a combination of demagnetized and undemagnetized directions—a degree of selectivity they find unacceptable—and has misinterpreted certain published results. Furthermore, they criticize Theyer's micropalaeontological work by giving chapter and verse on a series of supposed misidentifications and miscorrelations.

In his altogether more sedate reply, which follows the critique, Theyer defends his palaeomagnetic work largely in terms of later demagnetization studies which seem to justify the application of his original techniques to the particular samples concerned. He thus sticks to his interpretation of stratigraphy, where appropriate, in terms of the Gauss–Gilbert, rather than the Brunhes–Matuyama, polarity pattern. As far as the micropalaeontology is concerned, Theyer argues the point on what he takes to be the most crucial of his supposed misidentifications and in the end begs to differ with Watkins *et al.* on the question of interpretation. But he also notes that, criticisms of method and competence apart, a fundamental problem still remains. Why is it that, in at least twelve widely separated cores from the south-west Indian Ocean *G. truncatulinoides* overlaps distinctive late Miocene–Pliocene foraminifer and radiolaria?

In his original article Theyer rejected reworking as an unreasonable explanation and instead proposed the appearance of *G. truncatulinoides* during the Gauss epoch. He now admits, however, that other explanations are possible and require testing. Thus, by implication, he agrees with Watkins *et al.* to the extent that "one should not lightly deny the conclusions from many years of painstaking effort by many people".

Growth of the Biochemical Literature

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Analyses of the biochemical literature show which journals now contribute most to the subject and provide a guide for librarians with a limited budget.

THE first primary journal in biochemistry was *Hoppe-Seyler's Zeitschrift für physiologische Chemie* founded in 1877. Hoppe-Seyler in his preface in the first issue pointed out that "Biochemistry . . . from its first natural and necessary analytical beginnings has grown into a science . . .". The subsequent increase in the number of biochemical periodicals reflects its growth as a specialized area of knowledge.

Data compiled in the *World List of Scientific Periodicals* (fourth ed. and 1968 supplement) show that the number of biochemical journals increased from four in 1900 to twenty-three in 1920, seventy-seven in 1940, 201 in 1960 and 262 in 1968. Though exact figures since 1969 are not available, it is likely that the number of biochemical journals has continued to grow exponentially; and among new journals to appear many are devoted to subfields like biophysics, molecular biology, neurochemistry, comparative biochemistry, biochemical pharmacology and so on to which interest has shifted. As a result of all this most biochemists find themselves buried amidst piles of unread papers. To make matters more difficult, large numbers of papers of biochemical importance continue to appear in general science periodicals and in specialist periodicals in allied areas like microbiology and physiology. It has therefore become an essential need of the working biochemist as well as librarians and documentalists serving biochemical institutions to have an objective means of assessing the relative importance of the scientific periodicals now being published, from the point of view of their content of literature relevant to biochemistry. In particular it would help librarians of biochemical research institutions to utilize their budgets to the best advantage and to ensure that the research worker is spared the labour of wading through publications of relatively little value. The ranking list for biochemistry compiled by Henkle in 1938 is outdated and I have therefore reranked biochemical journals on the basis of citations in the *Annual Review of Biochemistry* for the years 1968, 1969 and 1970 (ref. 1).

Here "serials" has been taken to denote only publications brought out in different parts intended to be published for an indeterminate time, generally at regular intervals of not more than 1 year. Proceedings of international congresses, colloquia, meetings and so on were considered serials in case a permanent organization existed for arranging their publication, otherwise they were classed as books. Monographs of learned societies were also classed as books unless they were published in a numbered sequence. Serial annual publications devoted to recent advances, progress series and non-primary serial publications consisting of review articles were grouped together separately for purposes of analysis. Citations of abstracting journals were not counted with the serial citations in case the names of primary journals in which the original articles appeared

had been given. All citations found at the end of the articles in the *Annual Review*, within the texts of the articles and in footnotes were counted. Unpublished data, personal communications, theses and dissertations, reports, papers in press or awaiting publication were noted separately and treated as non-serial citations. Overall ranking was made on the basis of the grand total of citations of the various titles cited.

Primary journals accounted for 86.4% of all citations counted, non-primary serials for 4.7% and non-serial publications for 8.9%. Serial publications are thus the chief sources of current ideas and techniques of biochemistry. Of the non-serial publications, books and monographs accounted for 4.2% of all citations, and irregular conference/symposium proceedings 1.7%. Among publications other than primary journals, non-serial publications (8.9% of all citations) seemed to be of greater importance than non-primary serials (4.7%). The reasons for this may be the revival of the practice of reporting original results in monographs and the increasing number of publications reporting the proceedings of scientific conferences where results of current interest are presented and discussed.

Among primary journals 533 titles covering 16,276 citations were noted in my study, but of these 453 had been referred to less than twenty-one times, that is, with less than 1% of the frequency of the most cited periodical, and together accounted for only 9.3% of all the citations of primary journals. The latter were therefore excluded from our ranking list of primary journals which comprises eighty titles. The first thirty of these titles are listed in Table 1.

Henkle's 1938 list² comprised only thirty-seven titles. On comparison of the present list with Henkle's, we find that *J. biol. Chem.* has retained its top position, and *Nature* improved its position from 12 to 6, *Science, N.Y.* from 23 to 9, *J. exp. Med.* from 25 to 19, *J. clin. Invest.* from 26 to 24, *J. Biochem., Tokyo* from 32 to 16, *J. Pharmac. exp. Ther.* from 35 to 29 and *Endocrinology* from 36 to 22. The reason for the rise in ranking of

Table 1 First Thirty Titles in Ranking List for Biochemistry

(1) <i>J. biol. Chem.</i>	(15) <i>Eur. J. Biochem.</i> (formerly <i>Biochem. Z.</i>)
(2) <i>Proc. natn. Acad. Sci. U.S.A.</i>	(16) <i>J. Biochem., Tokyo</i>
(3) <i>Biochim. biophys. Acta</i>	(17) <i>Virology</i>
(4) <i>J. molec. Biol.</i>	(18) <i>J. Cell Biol.</i> (formerly <i>J. biochem. biophys. Cytol.</i>)
(5) <i>Biochemistry</i>	(19) <i>J. exp. Med.</i>
(6) <i>Nature</i>	(20) <i>J. Lipid Res.</i>
(7) <i>Biochem. biophys. Res. Commun.</i>	(21) <i>J. Immun.</i>
(8) <i>Biochem. J.</i>	(22) <i>Endocrinology</i>
(9) <i>Science, N.Y.</i>	(23) <i>Lipids</i>
(10) <i>Archs Biochem. Biophys.</i> (formerly <i>Archs Biochem.</i>)	(24) <i>J. clin. Invest.</i>
(11) <i>J. Am. chem. Soc.</i>	(25) <i>Can. J. Biochem.</i>
(12) <i>Fedn. Proc. Fedn. Am. Soc. exp. Biol.</i>	(26) <i>C.r. hebdom. Séanc. Acad. Sci., Paris</i>
(13) <i>J. Bact.</i>	(27) <i>Proc. Soc. exp. Biol. Med.</i>
(14) <i>Cold Spring Harb. Symp. quant. Biol.</i>	(28) <i>Ann. N.Y. Acad. Sci.</i>
	(29) <i>J. Pharmac. exp. Ther.</i>
	(30) <i>Hoppe-Seyler's Z. physiol. Chem.</i>

these journals is to be sought in two features of current biochemical research—its increasing significance to fundamental biological knowledge wherefore more space is devoted to it in journals of general science and the shifting of interest to special subfields like biochemical pharmacology, endocrinology and immunochemistry. *Biochem. Z.* (now *Eur. J. Biochem.*), *Biochem. J.*, *J. Am. chem. Soc.* and *C.r. hebdom. Séanc. Acad. Sci., Paris* ranking 2, 3, 9 and 16 in Henkle's list have gone down to positions 15, 8, 11 and 25, whereas Hoppe-Seyler's *Z. physiol. Chem., Am. J. Physiol., Proc. Soc. exp. Biol. Med., Chem. Ber., J. Physiol., Lond., Helv. chim. Acta, J. chem. Soc., J. gen. Physiol., Bull. Soc. Chim. biol.* (now *Biochimie*), *Proc. R. Soc. B.* and *Lancet* ranking 4, 5, 6, 7, 10, 13, 15, 17, 18, 24 and 28 have shifted to even lower positions—30, 49, 27, 66, 71, 62, 44, 32, 52, 34 and 44. Fourteen journals in Henkle's list among which are *C.r. Séanc. Soc. Biol., Klin. Wschr., J. Nutr., Liebigs Annln Chem., Pflügers Arch. ges. Physiol., Arch. exp. Path. Pharmac. and Naturwissenschaften* do not find a place in the present list. These changes may have been caused by the enormous expansion that has taken place in serial publications devoted to biochemistry and to its special branches, and the steady decrease in the importance of non-English scientific periodicals since the compilation of Henkle's list. As predicted by Sengupta⁸ in an earlier publication many post-war journals like *Biochim. biophys. Acta, J. molec. Biol., Biochemistry* and *Biochem. biophys. Res. Commun.* occupy a high position in the list and other more recently established journals like *Virology, J. Lipid Res., J. Neurochem., Tetrahedron Lett., Biophys. J., Life Sci., Biochem. Pharmac., FEBS Lett., Analyt. Biochem., Immunochemistry*, and *Molec. Pharmac.* have already found a place in it.

Table 2 shows an analysis of citations of the eighty journals in my ranking list according to subject classification. The preponderance of citations of journals exclusively devoted to biochemistry indicates the established status of biochemistry as a distinct scientific discipline. The high frequency of citations of journals of general science brings out the impact of biochemistry, particularly molecular genetics, on the culture of science today. Journals of chemistry account for only 6.9% of the citations, which may be because most chemical journals no longer have a section devoted to biochemistry. Table 2 also brings out the decrease in citations of physiological and medical journals, indicating perhaps a reversal of the historic inter-relationship between the disciplines of physiology and medicine and biochemistry, with the ideas and methodology of biochemistry determining the directions of physiological research.

Other findings were that English is now the principal medium of communication in biochemical research, 75% of the titles in my ranking list being English language publications. Multilingual journals, with their texts in

Table 2 Analysis of Citations According to Subject Classification of Periodicals

No.	Subject classification	No. of titles in ranking list	% of total citations
(1)	Biochemistry	20	55.05
(2)	Science (general)	6	19.19
(3)	Chemistry	17	6.91
(4)	Biological sciences (general)	7	5.60
(5)	Microbiology	7	4.86
(6)	Physiology	5	1.80
(7)	Cell biology	3	1.61
(8)	Medicine (specialities)	3	1.02
(9)	Experimental medicine	1	0.91
(10)	Pharmacology	3	0.77
(11)	Medicine (general)	3	0.71
(12)	Genetics	2	0.59
(13)	Experimental biology and medicine	1	0.50
(14)	Plant physiology	1	0.35
(15)	Psychology	1	0.14
Total:		80	100

English, French and German, come next (15% of the titles) although papers in English predominate in these also. Considering the distribution by country of the journals in the list, 64.5% of the journals were published from the United States and 19.7% from Britain. An interesting feature is that though fewer journals in the list are published from the Netherlands than from Germany, citations of the Netherlands journals (9.6%) far exceed those of German ones (2.9%). This is possible because of the high ranking of *Biochim. biophys. Acta*, published in the Netherlands.

An analysis of the number of citations in relation to size of journal and average length of the papers published is presented in Table 3. Analysis of data for the first ten journals in the list yields two parameters D/A and D/C which are indexes of the scientific interest of a journal corrected for its overall size (number of words in a year) and the scientific value of the papers in relation to the compactness of presentation. The parameter D/A , indicating the proportion of papers of most significance to biochemical knowledge, is highest for *Proc. natn. Acad. Sci. U.S.A.*, although it ranks second in my ranking list. Judged by this parameter, the significant information content of *Biochim. biophys. Acta* is relatively low, in spite of its high ranking, an indication perhaps of undue bulk. This parameter may be misleading, however, when applied to journals of broader scientific coverage like *Proc. natn. Acad. Sci. U.S.A.*, *Nature*, and *Science, N.Y.*, for the formula does not have a built-in correction for the variable proportion of papers of non-biochemical interest in such journals. For example, the significance of papers in *Proc.*

(continued on page 118)

Table 3 Analysis of Number of Citations in Relation to Size of Journal and Average Length of the Papers Published

Name of journal	No. of papers published during the year 1969 (A)	Total No. of words in the year 1969 ($\times 10^{-4}$) (C)	No. of citations noted for 1969 (D)	No. of citations per paper published (D/A)	No. of citations in relation to overall size of volumes published during the year (D/C)	Position in ranking list
<i>Biochem. biophys. Res. Commun.</i> , 34 to 37 (1969)	592	68.6	316	0.534	4.6	7
<i>Proc. natn. Acad. Sci. U.S.A.</i> , 62 to 64 (1969)	597	144	487	0.816	3.4	2
<i>J. molec. Biol.</i> , 39 to 46 (1969)	385	105	285	0.740	2.7	4
<i>J. Biol. Chem.</i> , 244 (1969)	902	382	729	0.808	1.9	1
<i>Biochem. J.</i> , 111 to 115 (1969)	982	184	255	0.260	1.4	8
<i>Archs Biochem. Biophys.</i> , 129 to 135 (1969)	532	143	167	0.314	1.2	10
<i>Biochim. biophys. Acta</i> , 171 to 195 (1969)	1,878	407	494	0.263	1.2	3
<i>Biochemistry</i> , 8 (1969)	732	327	309	0.422	0.9	5
<i>Nature</i> , 221 to 224 (1969)	2,297	335	256	0.111	0.8	6
<i>Science, N.Y.</i> , 163 to 166 (1969)	1,209	369	202	0.167	0.5	9

Histidinaemic Mutant in the Mouse

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A balance defect seen in a histidinaemic strain of mouse "segregates" abnormally, suggesting teratogenic effects of maternal metabolism. This effect may be analogous to certain human disorders.

IN a screening programme, designed to find mouse homologues of human inborn errors of metabolism, we have discovered a mutant which appears to be closely similar to human histidinaemia. Recent reports^{1,2} show that the incidence of this condition in man is as high as phenylketonuria. The unusual aspect of our findings is the relationship of a classical genetic block in histidine metabolism to a behavioural defect which is dependent on the maternal genotype. This type of interaction may play a part in a number of little understood human disorders.

Histidine and Imidazole Concentrations

As a result of a routine screening of mouse mutants for various biochemical parameters, including amino acids, a stock from Cambridge was received in Edinburgh and some of the offspring were found to have high levels of histidine in urine, liver, plasma and brain. There also occurred in this stock some animals having a balance defect which presented itself as circling and head tilting^{3,4}. By an appropriate crossing programme, based on urine histidine levels, a "true breeding" high histidine stock was obtained. The result of crosses of this stock to a normal strain (F_1) and an intercross (F_2) is given in Fig. 1 in terms of the liver histidine levels. Fig. 1c in particular indicates that the "high histidine" character is inherited as a single autosomal recessive. We designate the allele by the symbol *his*.

Pooling observations from many segregating matings (such as in Fig. 1c), a ratio of normal/high, not significantly differing from 3:1 was obtained (Table 1). There is no sex difference in this ratio.

Table 1 The 3:1 Segregation of Normal and High Tissue Histidine Concentrations from *his*/+ ♀ × *his*/+ ♂ Matings

	No. of offspring		χ^2	<i>P</i>
	Normal	High		
♀♀'s	81	24	0.257	NS
♂♂'s	77	27	0.0256	NS
All	158	51	0.0399	NS

Comparisons of histidine values in five tissues showed no exceptions to the classification of the phenotypes; the differences in adult mice between high and normal were at least 10-fold except for skin (Table 2).

The Cambridge stock was derived from wild trapped mice in Peru in 1962 (ref. 4). Introduction of the C57Bl/6J inbred line at various stages of the crossing programme indicated that

the normal allele in this line did not differ from the normal Peru in the segregation ratio.

The accumulation of large quantities of histidine suggests that one of the steps in this catabolism is blocked (Fig. 2). Measurements of histidase (histidine ammonia lyase EC 4.3.1.3) showed that all *his*/*his* animals had low activities (0.0–0.05 $\mu\text{mol min}^{-1} \text{g}^{-1}$ liver at 30° C) whereas the other genotypes (*his*/+ and +/+) fell in a range of higher activities (0.08–0.37). There was no significant difference in urocanase between the genotypes.

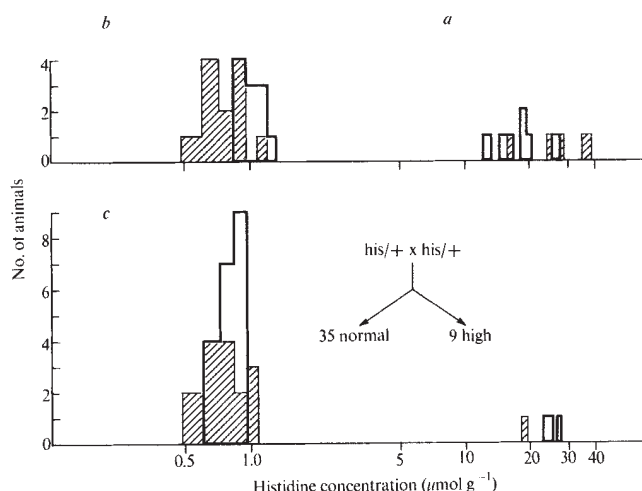


Fig. 1 Frequency distribution of liver histidine concentrations. *a*, Offspring of the true breeding "high histidine" stock. *b*, Offspring of a cross of (*a*) animals with normal C57 (F_1). *c*, Offspring of an intercross (F_2) of animals of (*b*). Of the nine "high" animals only four were killed and their liver values determined. The five remaining were identified from their urine values (see Table 2). Hatched areas: female; unhatched areas: male.

As a result of the low histidase activities causing high histidine levels, the concentrations of the derived imidazoles are raised. This is particularly so in the urine (Table 3). As expected, these high concentrations usually have positive reactions with ferric chloride ('Phenistix', Ames Co.). As with histidine, column chromatography of the imidazoles allows an unambiguous classification of mutant homozygotes.

Table 2 Concentrations of Histidine in Various Tissues at 7 Weeks

Tissue	Mean value and s.e. of histidine High (<i>his</i> / <i>his</i>)	Mean value and s.e. of histidine Normal (+ / +)	Ratio = $\frac{H}{N}$
Liver *	21.16 ± 0.94	0.97 ± 0.025	21.8
Brain *	2.10 ± 0.19	0.17 ± 0.007	12.2
Plasma †	3.29 ± 0.35	0.12 ± 0.005	28.7
Urine †	0.86 ± 0.16	0.04 ± 0.003	21.5
Skin *	0.49 ± 0.08	0.23 ± 0.050	2.1

All determinations were carried out by column chromatography and ninhydrin reaction.

* For brain, liver and skin the units are $\mu\text{mol g}^{-1}$.

† For plasma and urine the units are $\mu\text{mol ml}^{-1}$.

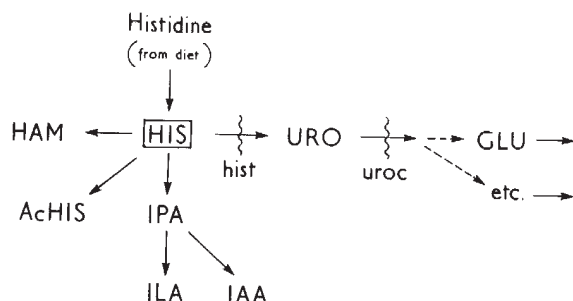


Fig. 2 Pathway of histidine catabolism. HAM: histamine; AcHIS: N-acetylhistidine; HIS: histidine; URO: urocanic acid; IPA: imidazole pyruvic acid; ILA: imidazole lactic acid; IAA: imidazole acetic acid; GLU: glutamic acid; hist: histidase; uroc: urocanase.

Histidase Activities

In the mouse⁵ and in man⁶ it has been shown that, where the primary enzymatic lesion is known, recessivity at the metabolic or clinical level is associated with intermediate values at the enzyme level in heterozygotes. This phenomenon has been used as one criterion to establish whether an enzyme is the site of action of a mutant gene^{7,8}.

Table 3 Concentrations of Imidazole Derivatives in Urine

Imidazole derivative	Mean value of imidazole derivatives High (<i>his/his</i>)	Normal (+/+)	Ratio = $\frac{H}{N}$
Imidazole pyruvic acid + acetyl histidine*	3.19 ± 0.87	0.11 ± 0.03	29.0
Imidazole lactic acid	1.21 ± 0.17	0.07 ± 0.02	17.3
Imidazole acetic acid	1.29 ± 0.19	0.05 ± 0.01	25.8

The values given have the units $\mu\text{mol ml}^{-1}$.

All determinations were carried out by column chromatography and Pauly reaction. The analytical system was similar to that described by Wadman *et al.*¹⁸.

* In our buffer system these two substances are not completely resolved. The values reported are the sum of both.

Additional heterozygous matings (*his*/+ $\times$ *his*/+) were therefore constructed for enzyme determinations. The enzyme activities of the offspring are shown in Fig. 3. As we had already established that normal females have up to twice the activities of normal males (compare also rats⁹), male and female values are shown separately. Segregation of these animals into three classes can be easily seen in the females (Fig. 3d). The numbers in the classes do not differ significantly from 1:2:1 (15:44:16; $\chi^2=2.28$, not significant). The mean of the intermediate group is 45% of the mean of the high group. Known heterozygous females (from *his*/*his* $\times$ +/+ matings) always fell into the intermediate group (Fig. 3c). The three enzyme classes are therefore consistent with the genotypes *his*/*his*, *his*/+ and +/+ respectively. The segregation into classes of enzyme activity is not so clear for males (Fig. 3a, b). This male-female difference is not yet understood and is under further investigation. The males, however, can be scored reasonably well for three classes on a within-litter basis, when the numbers do not differ significantly from 1:2:1. It therefore seems certain that the *his* gene is autosomal and this is consistent with it being an allele at the structural locus for the enzyme histidase.

Relation between Histidine and Histidase

The available values for liver enzyme activities and histidine levels of individual mice make it possible to establish the role of histidase in controlling the concentration of its substrate. This is shown in Fig. 4 where the two sets of values are plotted

irrespective of age, sex or genotype. It can be seen that the histidine concentrations are "buffered" down to histidase activities of approximately 0.10 units and only begin to rise significantly at values below this. When the value of the enzyme is substantially zero, the level of histidine is, presumably, controlled by the activities of other catabolic steps. This is being further investigated. Such behaviour is expected from the general analysis of enzyme systems^{10,11} and is an example of how quantitative variation at the enzyme level can generate dominance at the metabolite, flux or physiological level.

The Balance Defect

The *his* mutant was found in a stock derived from wild mice. The stock was investigated because some animals appeared which showed a balance defect (BLA). This is characterized by some or all of the following: circling behaviour, head tilting, deafness, inability to swim, lack of disorientation after spinning, and poor maze learning.

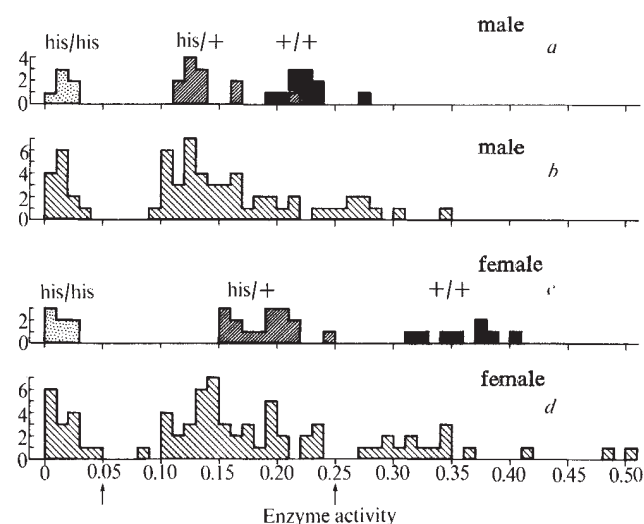


Fig. 3 Histidase activities in livers of different genotypes. Males (a, b) and females (c, d) are shown separately. a and c, Identified genotypes, either tested or constructed; b and d, segregating offspring from *his*/+ $\times$ *his*/+ matings, genotype not identified. Histidase (EC 4.3.1.3) activity was determined by the appearance of urocanic acid at 277 nm. The assay system was 65 mM phosphate buffer (pH 9.4), 5 mM reduced glutathione, 133 mM L-histidine HCl, 0.05 ml enzyme, final volume 1 ml. The liver was extracted in a Tri-R homogenizer 3:1 v/w with a solution which was 0.25 M sucrose, 1 mM EDTA sodium salt, 1 mM dithiothreitol, 50 mM Tris-HCl (pH 7.4). The homogenate was centrifuged at 60,000g_{av} and the supernatant used to assay histidase as $\mu\text{mol urocanate min}^{-1} \text{g}^{-1}$ at 30° C.

Before the presence of the *his* allele was known, selection for BLA established a colony with 80% incidence of this condition. The defect disappeared on outcrossing to AG/Cam and had a very low incidence in the first few backcrosses to the BLA stock. There was also variation in incidence between litters from the same mating. It was concluded from this that the balance defective character was controlled by an imperfectly penetrating recessive gene under non-genetic as well as polygenic control^{3,12}.

With the discovery of segregation at the *his* locus in derivatives of this stock received in Edinburgh a relationship between the three *his* genotypes and the balance defect was sought. It was immediately apparent that no direct relationship existed: BLA animals having either *his*/*his* or *his*/+ genotypes were found. Similarly, many animals with these genotypes were behaviourally normal.

A new explanation presented itself when the first doubly

homozygous matings were made (*his/his* mothers $\times$ *his/his* fathers). Most of the offspring showed the BLA characteristics. Similarly, in crosses with *his/his* mothers and $+/+$ fathers (C57) some animals were found with BLA characteristics although of a milder form (head tilting).

In contrast, no animals with BLA characteristics were obtained from $+/+$ mothers and *his/his* fathers; nor did *his/+* mothers and *his/+* fathers produce any offspring showing BLA although about a quarter of these were demonstrably *his/his*.

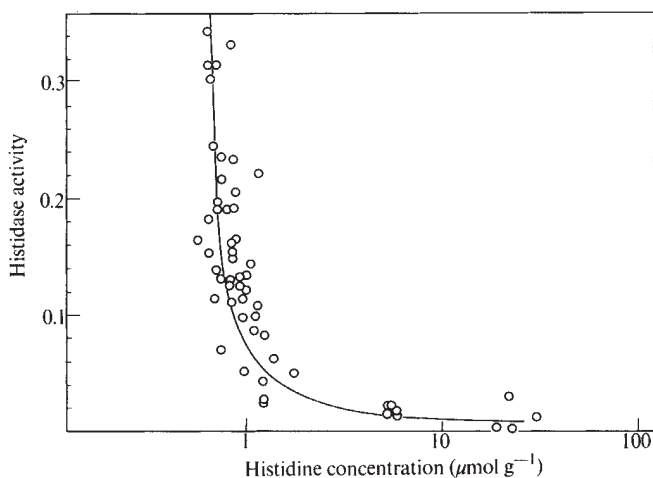


Fig. 4 Liver histidase and histidine. Enzyme activity measured in μmol urocanate $\text{min}^{-1} \text{g}^{-1}$ at 30°C as in Fig. 3.

This strongly suggests a maternal effect as the principal cause of the balance defect. It is proposed that the metabolic state of the pregnant mother of *his/his* genotype is necessary for the appearance of BLA. It should be stressed that BLA offspring were obtained from *his/his* mothers whether they themselves were BLA or not. In fact, because of the poor breeding performance of BLA females (whether *his/his* or not) most BLA offspring were born of normal (non-BLA) but *his/his* mothers. The severity of the condition, however, seems to be related to the genotype of the offspring; *his/his* offspring are more severely affected than those that are *his/+*.

Litter Size, Parity and Vestibular Damage

Further evidence for a maternal effect of *his/his* mothers comes from an examination of the numbers of surviving offspring. It has been shown that *his/his* animals from *his/+* mothers have the same survival as their littermates (see Table 1 showing a 3:1 ratio). The genotype of the offspring therefore has no effect on survival. The mothers' genotype, however, affects the number of pre-natal deaths, being about 30% higher in *his/his* compared with *his/+* mothers. Further differential lethality is shown up to the age of weaning. Animals that reach weaning seem to have as good a chance of surviving as normal animals. This difference can therefore be attributed to the influence of the maternal metabolic environment.

There seem to be, however, influences other than maternal ones or offspring genotype which affect the expression and the frequency of BLA. There is heterogeneity between different matings. The proportion of severe/mild/normal offspring from *his/his* $\times$ *his/his* matings ranges from 0/4/5 to 9/0/0. At the moment it is not known whether this heterogeneity is due to sampling or has a genetic or environmental basis.

Another factor affecting the frequency of BLA is parity³. There seems to be a steady decline in the proportion of surviving BLA animals from first to later litters. In one series the % BLA offspring (from non-BLA mothers) was 62% in the first litter, falling to about 30% in the fourth and later ones.

There is thus firm evidence that the maternal environment provided by histidinaemic mothers affects the development of the offspring, the balance defect being the most obvious manifestation. Its inheritance will therefore not fit any simple Mendelian hypothesis which attempts to attribute the condition to the action of a gene carried by the individual.

Genetic balance defects in the mouse have often been associated with anatomical abnormalities of the inner ear¹³ but these segregate normally and are attributed to some consequence of the individual's genotype. There are two reports of mutants which showed "abnormal segregation" similar to the BLA condition. These are *zig zag*¹⁴, which had irregular movements and deafness, and *careener*¹⁵, which showed unbalanced locomotion. Examination of the inner ear of *zig zag* showed that the horizontal semicircular canal was thin or broken. *Careener* showed otolith defects and we are investigating this strain. A number of ears from BLA animals were kindly examined by Dr Mary Lyon and she reports various degrees of shortening of the posterior vertical canal and the crus commune, and absence of otoliths in either or both ears. We therefore conclude tentatively that the maternal metabolic state produces a teratogen which affects some developmental process *in utero* with consequent permanent impairment of the inner ear function. A histological examination of BLA (*his/his*) brains and spinal cords was carried out by Dr H. Fraser who reports no evidence of histological abnormality in overall cellular morphology and that the nerve fibres and their myelin sheaths were intact. Whether other neurological functions are affected remains to be determined.

Histidinaemia in man appears to have the same enzymatic basis as in our mutant (see for example Auerbach *et al.*¹⁶ and Neville *et al.*¹⁷). The metabolic consequences—high histidine and imidazole derivatives—are very similar in the two species. A behavioural abnormality often associated with human histidinaemia is a speech defect and mental retardation. As with the *his*/BLA syndrome, a considerable proportion of human subjects are now known who are histidinaemic but behaviourally normal. Unlike the case in the mouse, however, no evidence of the involvement of a maternal effect is reported for man. In spite of the considerable reservations which one must have in associating the behavioural aspects in the two species, the further elucidation of biochemical, physiological and nutritional aspects in the mouse may throw light on the aetiology of the human disorder (G. B. and H. K., in preparation).

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- ¹ Raine, D. N., Cooke, J. R., Andrews, W. A., and Mahon, D. F., *Brit. Med. J.*, **3**, 7 (1972).
- ² Lott, I. T., Wheelden, J. A., and Levy, H. L., *Develop. Med. Child. Neurol.*, **12**, 596 (1970).
- ³ Wallace, M. E., *Mouse News Lett.*, **42**, 20 (1970).
- ⁴ Wallace, M. E., *Environ. Pollut.*, **1**, 175 (1971).
- ⁵ Coleman, D. L., *Arch. Biochem. Biophys.*, **96**, 562 (1962).
- ⁶ Harris, H., *The Principles of Human Biochemical Genetics* (North-Holland, Amsterdam and London, 1970).
- ⁷ Paigen, K., in *Enzyme Synthesis and Degradation in Mammalian Systems* (edit. by Rechigl, M.) (Karger, Basel, 1971).
- ⁸ Bulfield, G., *Genet. Res.*, **20**, 51 (1972).
- ⁹ Noda, K., and Nakagawa, H., *Endocrinology*, **90**, 207 (1972).
- ¹⁰ Kacser, H., and Burns, J., *Quantitative Biology of Metabolism*, 11 (Third International Symposium, Biologische Anstalt Helgoland, 1968).
- ¹¹ Kacser, H., and Burns, J., in *Symp. Soc. Exp. Biol.*, **27**, 65 (1973).
- ¹² Wallace, M. E., *Mouse Newsletter*, **48**, 25 (1973).
- ¹³ Sidman, R. L., Green, M. C., and Appel, S. H., *Catalog of the Neurological Mutants of the Mouse* (Harvard University Press, Cambridge, Massachusetts, 1965).
- ¹⁴ Lyon, M. F., *Genet. Res.*, **1**, 189 (1960).
- ¹⁵ Chai, C. K., and Chiang, M. S. M., *Genetics*, **47**, 435 (1962).
- ¹⁶ Auerbach, V. H., DiGeorge, A. M., Baldrige, R. C., Tourtellotte, C. D., and Brigham, M. P., *J. Pediatr.*, **60**, 487 (1962).
- ¹⁷ Neville, B. G. R., Bentovim, A., Clayton, B. E., and Shepherd, J., *Arch. Dis. Child.*, **47**, 190 (1972).
- ¹⁸ Wadman, S. K., De Bree, P. K., Van der Heiden, C., and Van Sprang, F. J., *Clin. Chim. Acta*, **31**, 215 (1971).

Bubble Model of Extragalactic Radio Sources

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The components of radio sources are identified with bubbles of relativistic plasma rising through the hot gas which produces the X-ray emission from clusters of galaxies. Continuous release of energy in the nucleus of a galaxy will lead to the formation of two such bubbles, moving in opposite directions along the axis of rotation.

MANY extragalactic radio sources are associated with giant elliptical galaxies and have radio source structures consisting of two components, small compared with their separation, on either side of the galaxy¹. It is generally assumed that the large amount of energy ($\sim 10^{52}$ J) required to form the source components originated in the nucleus, which often exhibits continuing violent activity²; models of radio galaxies³ must therefore account for the efficient transport of this energy from the nucleus to the outer components. The principal difficulties in formulating such models arise from the highly directional nature of this process and from the efficient confinement of the components necessary to prevent the catastrophic energy losses which would result from adiabatic expansion.

In the past, the possibility that the components of these sources may be in pressure equilibrium with a hot intergalactic gas has been neglected, because it has been assumed that the components are located within the general intergalactic medium upon the density and temperature of which stringent limits ($T < 10^6$ K, $n_e < 10 \text{ m}^{-3}$) can be set from observations of the X-ray background⁴. But many of the radio sources are known to be in clusters of galaxies and Uhuru has now detected extended X-ray sources close to the centres of the nearer rich clusters⁵, implying the presence of a large cloud of hot gas in each of these clusters if the X-rays are attributed to thermal bremsstrahlung.

Here we re-examine the "external pressure" confinement models for radio sources and construct a model in which the X-ray emission is due to a hydrostatic atmosphere of hot gas trapped in the gravitational potential well of the cluster. The pressure of a gas dense enough to account for the X-ray emission is sufficient to contain even the most powerful radio sources. We then show that the radio components behave like bubbles which rise through the ambient gas. Considerations of how such bubbles form in galactic nuclei lead to a simple picture in which two bubbles escape in opposite directions if the galaxy is at the centre of a cluster and thus stationary with respect to the hydrostatic atmosphere. If the active galaxy is offset towards the edge of the cluster, its motion through the gas must be taken into account and the structure of the resulting radio source will be complex.

Confinement by Intergalactic Gas Pressure

If the components are to be confined, the external gas pressure must balance the internal pressure, for which a lower limit may be found by taking the minimum energy densities of magnetic field and relativistic particles required to produce the non-thermal radio emission. The external pressure required varies widely from one source to another: for the compact components of a large double source such as Cygnus A a gas pressure of $\sim 10^{-9} \text{ N m}^{-2}$ is necessary^{6,7}, but for weaker, more extended sources such as 3C66 or 3C465 a much lower pressure of $\sim 10^{-13} \text{ N m}^{-2}$ suffices.

We assume that the gas in the cluster forms an atmosphere in hydrostatic equilibrium and it is therefore necessary to know the shape of the gravitational potential well of a cluster of galaxies with a massive elliptical galaxy at its centre. We further assume that the hot gas has sufficient internal energy just to fill this potential well. This energy could result from gravitational infall or from internal sources of heat such as the random motions of galaxies or galactic explosions. The shape of this potential well depends on the mass distribution which, for the central elliptical galaxy, we have derived from the luminosity distribution $L(R)$ on the assumption of a constant mass/light ratio. De Vaucouleurs⁸ finds a relation $L(R) \propto \exp(-3.33 (R/R_e)^{1/4})$, where R_e is the semi-major axis of the ellipse that contains half the light, whereas Hubble⁹ gives $L(R) \propto B/(R+A)^2$ where B and A are scale parameters. We have used a potential function derived from de Vaucouleurs's law outside R_e and continued this to the centre using Hubble's law, because the former provides a better fit to the observations at large distances but the latter is better near the centre¹⁰. Although the form of the mass distribution in a cluster of galaxies is not well known, there is some evidence^{10,11} that it is similar to that of elliptical galaxies. We have therefore used the same form of potential well to describe a cluster.

For this composite potential well we have constructed possible atmospheres by assuming that the gas is well mixed and in hydrostatic equilibrium. The characteristics of the atmosphere are only weakly dependent on the form of luminosity distribution used to derive the potential well. For a cluster of mass $M = 4 \times 10^{15} M_\odot$ and $R_e = 2 \text{ Mpc}$ (such as the Coma cluster¹¹) the temperature of the gas at the centre is $\sim 10^9 \text{ K}$ and the effect of the galaxy ($M \sim 3 \times 10^{13} M_\odot$; $R_e \sim 50 \text{ kpc}$) is to increase this temperature by $\sim 2 \times 10^8 \text{ K}$. Clearly, at the centre of a cluster the temperature of the gas is dominated by the cluster rather than the galaxy and, for the purposes of containing radio sources, we may consider that the galaxy is surrounded and permeated by isothermal gas at a temperature of $\sim 10^9 \text{ K}$. The gravitational acceleration, on the other hand, in the vicinity of the galaxy is dominated by the galaxy itself (Fig. 1) rather than the cluster.

The most severe containment problems are posed by Cygnus A, whose components have the highest observed energy density of any radio component outside the nucleus of a galaxy. It is

significant that this powerful radio source has been reported to lie in a cluster of richness 3 on Abell's scale¹². For such a cluster we assume $M=10^{16}M_{\odot}$, $R_c=2$ Mpc and thus the central temperature 2×10^9 K. To confine the radio components the gas pressure must be at least 10^{-9} N m⁻², implying a gas density of 8×10^3 m⁻³. If the hot gas cloud is to extend as far as both components of the source it must have a diameter of at least 200–300 kpc. Such a cloud is only directly observable through its X-ray emission and, for a diameter of 300 kpc, this would amount to $\sim 2 \times 10^{37}$ W in the 2 to 10 keV band, an order of magnitude less than the reported luminosity⁵ in this band of 1.2×10^{38} W (taking $H=50$ km s⁻¹ Mpc⁻¹). Thus sufficient hot gas to contain the radio components could certainly exist in this cluster.

Bubbles of Relativistic Plasma

Having established that pressure equilibrium is possible for even the most intense radio source components, we propose a model for radio sources in which the brightest components are identified with bubbles of a very hot or relativistic plasma produced in the nucleus of the galaxy. If a bubble of this plasma detaches from the nucleus it will rise through the galaxy under the influence of buoyancy forces, being less dense than the surrounding cooler gas. If the active galaxy is not at the dynamical centre of the cluster it will, in general, have some systematic motion through the gas. Because the gas is so hot that it is not gravitationally bound to the galaxy the bubble will be swept out of the galaxy by ram pressure. Thus, if the gravitational acceleration due to the galaxy is to determine the motion of the bubble, this systematic motion must be small. This is likely to be the case if the central, giant elliptical galaxy is responsible for the radio source and we shall assume this in what follows.

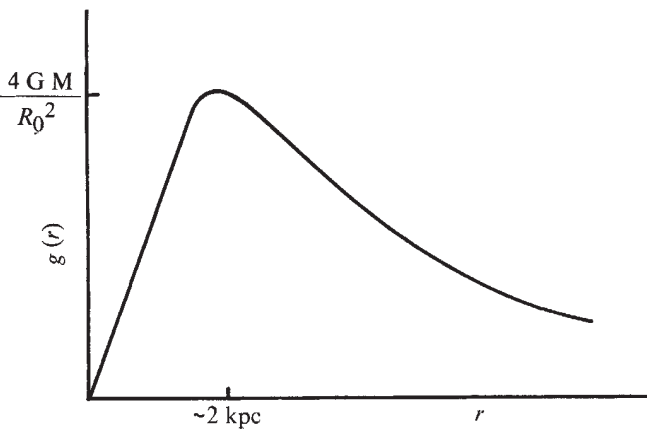


Fig. 1 Gravitational acceleration as a function of radius for an elliptical galaxy.

The behaviour of a large bubble rising under gravity at high Reynolds number in an incompressible fluid has been investigated empirically^{13,14} and it is found that a quasi-equilibrium is quickly established, in which the bubble assumes the form of a nearly perfect spherical cap followed by a highly turbulent wake (Fig. 2). The assumption of a high Reynolds number is valid because even a very weak magnetic field will ensure that the mean free path of the particles is much smaller than any scale of interest.

By equating the buoyancy forces to the ram pressure it is found that the bubble rises with terminal velocity $V_T \sim (rg)^{1/2}$ where r is the radius of the cap and g is the acceleration due to gravity. This velocity will in general be much less than the external sound speed V_s , because $V_s \sim (Hg)^{1/2}$ where H is the scale height of the pressure distribution which is of the same order as the size of the cluster.

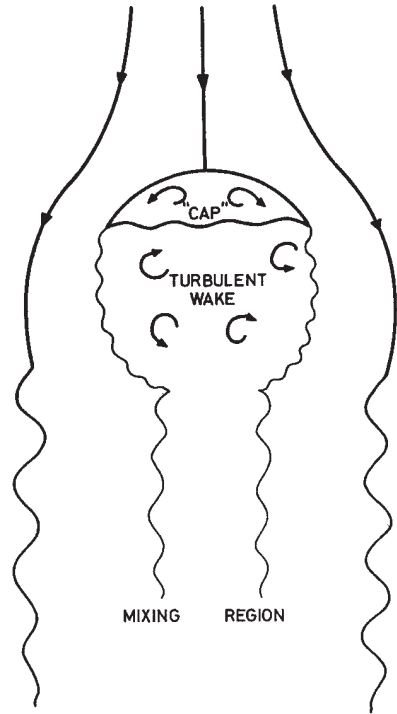


Fig. 2 Steady flow around cap bubble.

Formation of Bubbles in Galactic Nuclei

How could the continuous release of energy in the nucleus lead to the formation of two large detached bubbles? Explosions in galactic nuclei (such as supernovae) give rise to clouds of hot plasma which will quickly come into pressure equilibrium with the ambient gas. Thus, if the rate of release of energy (from whatever source) is constant, the net effect is that the volume of hot plasma increases at a constant rate and, as the internal sound speed is high, the whole volume maintains pressure equilibrium with the external gas.

If V is the volume of plasma produced in time t , a cavity of mean radius $r=(3V/4\pi)^{1/3}$ is formed. This cavity will be extended along the rotation axis, because in this direction the effective gravitational acceleration is greatest. If dV/dt is constant, the boundary between the two gases is decelerating ($\ddot{r} = -(2/9)rt^{-2}$). On the other hand, the hot plasma as a whole feels a net outward acceleration $g(r)$ due to buoyancy forces. Therefore, as the cloud expands, there is a time $t \sim (2r/9g)^{1/2}$ after which the buoyancy forces are dominant and the boundary suffers a Rayleigh-Taylor instability. The cavity will disrupt after a further time $\tau_{det} \sim (r/g)^{1/2}$ when perturbations of size $\sim r$ have developed. Because the cavity is already elongated, we expect two approximately equal bubbles of size r to detach and leave the nucleus along the rotation axis (Fig. 3).

We must distinguish between the cases when the time scale τ_{prod} for energy production is greater than or less than the detachment time τ_{det} of the bubble. In the former, with $\tau_{prod} > \tau_{det}$, a naive application of our argument would suggest that a stream of bubbles should be observed, all having roughly the same internal energy and radius. It is more likely, though, that the stream of bubbles will be disrupted because they follow in the turbulent wake of the first bubble. In the latter case, with $\tau_{prod} < \tau_{det}$, all the available energy will be carried outwards by two bubbles.

To estimate the time τ_{det} we use the form of $g(r)$ derived earlier (Fig. 1). Within the nucleus of the galaxy ($r < 2$ kpc) $g(r) \propto r$ and so the time required for a bubble to detach $\sim (r/g)^{1/2}$ is independent of the size of the bubble provided that this is less than 2 kpc. This time scale is 10^6 yr for a galaxy of mass $3 \times 10^{13} M_{\odot}$ and radius $R_c \sim 20$ kpc. Thus the rate of energy

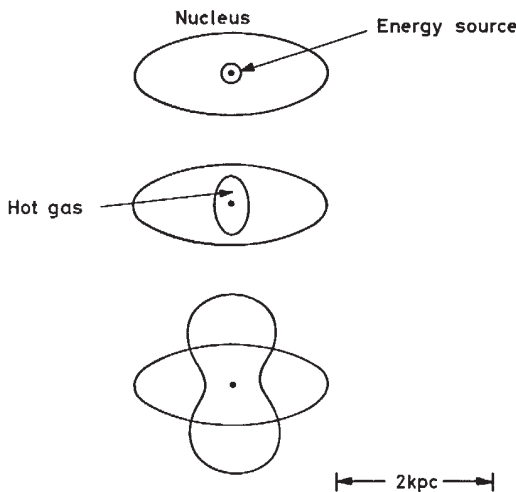


Fig. 3 Stages in the formation of a bubble in the nucleus of a galaxy.

supply required to produce the components of Cygnus A is $10^{46} \text{ J yr}^{-1}$ and it is interesting that this figure is similar to the rate of energy production observed in active galactic nuclei².

It seems feasible that this modest rate of energy production could result from a series of supernova events in the nucleus. If each supernova releases an energy $E = 10^{45} \text{ J}$ and mass $M = 5 M_{\odot}$ to the ambient hot gas in the nucleus, then the resulting supernova remnant differs radically from remnants in our own Galaxy (such as Cas A¹⁵). The external pressure is here large enough to cause the expansion to become subsonic before the blast wave stage is reached. The material and energy are dispersed by random motions, resulting in local heating of the plasma in the bubble. As this final stage is reached when the work done against the external pressure (P) is equal to the energy of the explosion, we may consider that a supernova contributes a volume $V = E/P$ at a density M/V . For a pressure of 10^{-9} N m^{-2} we find $V \sim 3 \times 10^{14} \text{ pc}^3$ and $T \sim 10^{10} \text{ K}$. To form the components of Cygnus A by this mechanism, a supernova rate of $> 10 \text{ yr}^{-1}$ for 10^6 yr is required.

Bubble Model of Cygnus A

We now discuss, with particular reference to Cygnus A, the interpretation of the structure and properties of extragalactic radio sources in terms of the model. We identify the low brightness extensions with the wake of a bubble; because the dynamics require the wake to have a similar radius to the bubble we take a bubble radius of 8 kpc for the components of Cygnus A. This is considerably larger than the sizes reported for the most compact components of the source^{6,7} and we shall attribute these components to localized "hot spots", due to turbulent vortices within the cap.

Before we can make a detailed comparison between the model and the observations of Cygnus A we need to estimate the present velocity of each bubble and hence the age of the source. If we assume that the mass of the galaxy is $3 \times 10^{13} M_{\odot}$ then at a distance of 90 kpc the terminal velocity is 350 km s^{-1} . The mean velocity during the evolution of the source is larger than this and we estimate a velocity of 800 km s^{-1} , suggesting that the age of the source is 10^8 yr . We note the following consequences of the model:

(1) The cap contains a store of internal energy in the form of all the relativistic particles that were originally generated in the nucleus of the galaxy. To produce radio synchrotron emission there must also be a magnetic field, which can be generated by the turbulent shearing motions within the cap. The magnetic field will be greatest at the centre of the vortices in the cap and these we identify with the brightest radio components.

The lifetime of relativistic electrons radiating at 5 GHz in the magnetic field of $3 \times 10^{-8} \text{ T}$, the equipartition value for the most compact components, is 10^5 yr , much shorter than the estimated lifetime of the source. Therefore the compact components must be non-stationary phenomena in which the relativistic electrons are continually replenished. In the bubble model the relativistic electrons are distributed throughout the cap, but only those electrons trapped within the vortices radiate at the above high rate. As these electrons lose energy they may be replaced by the electrons filling the bulk of the cap. The radiation lifetime of the cap at 5 GHz is longer than the electron lifetime in the vortex by the ratio of the volume of the cap to the volume of the vortex; for the parameters quoted above and taking a diameter of 2 kpc for the most compact components^{6,7}, the lifetime of the cap is $5 \times 10^7 \text{ yr}$, comparable to the age of the source. As this process develops the cap radiates away some of its internal energy and, because it must be in pressure equilibrium with its surroundings, gradually shrinks in size.

(2) Magnetic field is also generated by the turbulence in the wake. Since the material of the wake is just the thermal gas of the cluster, there is no reserve of relativistic particles that produce radio emission. It is possible, however, that particles may be accelerated in the strong turbulent field by the mechanisms discussed by Tsytovich¹⁶. Under these conditions it is reasonable to expect radio emission with approximate equipartition of energy between turbulence, magnetic field and relativistic particles. A similar process may occur in the vortices of the cap and thus serve to replenish the supply of relativistic particles.

Because the magnetic field in the wake ($5 \times 10^{-9} \text{ T}$ is an average value) is much weaker than that in the vortices, the radiation lifetimes of the electrons are much longer though, for the higher energy electrons radiating in the brighter parts of the wake, these lifetimes are less than the age of the source. The source has a break in its integrated spectrum at 1 GHz (refs 6, 7) and because at this frequency the low brightness tails dominate the radio emission we associate this break with the radiation lifetimes of the electrons in the wake.

(3) The model predicts a gas density of $8 \times 10^3 \text{ m}^{-3}$ in the emitting regions of the wake. Differential Faraday rotation will produce depolarization at a wavelength λ where $l \sim 8.1 \times 10^5 \lambda^2 \int n_e B_{\parallel} dl$ (l = path length (kpc); $B_{\parallel}$ = line of sight component of magnetic field (T); n_e = external particle density (m^{-3})). If we assume that the gas is fully ionized and that the magnetic field is $5 \times 10^{-9} \text{ T}$ and has irregularities of scale $\sim 1 \text{ kpc}$, we expect depolarization at a frequency of 4 GHz. This is consistent with the high rotation measure observed in the source¹⁸.

(4) There is a region around the nucleus of size $\sim 5 \text{ kpc}$ in which strong line emission occurs, indicating a temperature of $2 \times 10^4 \text{ K}$ and electron density $n_e \sim 10^{10} \text{ m}^{-3}$ in localized regions¹⁹. This implies a pressure for this cool gas of $3 \times 10^{-9} \text{ N m}^{-2}$, closely similar to that derived above for the gas in the cluster.

Further Consequences

In our model, the properties of a radio source are determined solely by the size and shape of the gravitational potential well near the galaxy, the gas density in the cluster and the rate of energy production in the nucleus. The model therefore predicts some close correlations between the size and power of a radio source and the properties of the associated galaxy and cluster.

Radio sources must be located within the gas distribution of clusters of galaxies or inside the envelopes of large elliptical galaxies. We therefore expect that the sources with the highest energy densities are located in the richest clusters, where the gas pressure and thus the X-ray flux are presumably greatest. Further, the temperature of this gas is sufficiently high ($\sim 10^9 \text{ K}$) that these clusters should also be sources of hard (10–50 keV) X-rays.

Because the gas pressure varies little near the centre of a

cluster, the size of a radio source component (d) remains approximately constant as the separation from the nucleus (D) increases. This is in contrast to other models of radio sources, in which the components are supposed to expand as they evolve. As the bubble moves outwards, it slows due to the decreasing gravity. Thus the radio source spends most of its life far from the nucleus, with a large value of D/d .

As the components slow, the input of turbulent energy decreases. Because in this model the radio emission is closely related to the turbulence, the radio power will slowly decrease.

The radio emission will be polarized, because the turbulent magnetic field in the wake will be ordered on scales up to the size of the cap and thus the structure should be complex and cellular. The polarization of the cap will also be high but, because of the complexity of the vortex, it may be difficult to observe.

As the bubble rises it will oscillate from side to side with an amplitude $\sim r$ (refs 13 and 14). This means that the wake may appear at an angle to the line joining the brightest components and the nucleus. Such deviations are frequently observed (Harris, unpublished).

Some radio sources seem to be linked to the galaxy by long, broad ridges of low brightness emission¹⁷. As outlined above, these may be the remnants of secondary bubbles which have been shattered by the turbulent wake of the first.

We have so far only considered the strong double sources. However, the model is readily adapted to account for the structures of the more complex sources (such as Centaurus A, 3C66) that are found outside or at the edge of clusters. In sources outside clusters the bubbles soon arrive at regions of low pressure and thus expand. This will result in large scale, complex, low brightness structure. Complex sources will also

result when the active galaxy is not at the dynamical centre of its cluster.

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- ¹ Ryle, M., *Q. Jl R. astr. Soc.*, **11**, 429 (1970).
- ² Burbidge, G. R., *Ann. Rev. Astr. Astrophys.*, **8**, 369 (1970).
- ³ De Young, D., and Burbidge, G. R., *Comm. Astrophys. Space Phys.*, **5**, 21 (1973).
- ⁴ Longair, M. S., *Rep. Prog. Phys.*, **34**, 1125 (1971).
- ⁵ Giacconi, R., Murray, S., Gursky, H., Kellogg, E., Schreier, E., and Tananbaum, H., *Astrophys. J.*, **178**, 281 (1972).
- ⁶ Mitton, S., and Ryle, M., *Mon. Not. R. astr. Soc.*, **146**, 221 (1969).
- ⁷ Miley, G. K., and Wade, C. M., *Astrophys. Lett.*, **8**, 11 (1971).
- ⁸ Vaucouleurs, G. de, *Mon. Not. R. astr. Soc.*, **113**, 134 (1953).
- ⁹ Hubble, E., *Astrophys. J.*, **71**, 231 (1930).
- ¹⁰ Vaucouleurs, G. de, *Handbuch der Physik* (edit. by Flugge, S.), **53**, 311 (Springer-Verlag, Berlin, Göttingen, Heidelberg, 1959).
- ¹¹ Rood, H. J., Page, T. L., Kintner, E. C., and King, I. R., *Astrophys. J.*, **175**, 627 (1972).
- ¹² Matthews, T. A., Morgan, W. W., and Schmidt, M., *Astrophys. J.*, **140**, 35 (1964).
- ¹³ Davies, R. M., and Taylor, G. I., *Proc. R. Soc., A*, **200**, 375 (1950).
- ¹⁴ Wegener, P. P., and Parlange, J.-Y., *Ann. Rev. Fluid Mech.*, **5**, 79 (1973).
- ¹⁵ Gull, S. F., *Mon. Not. R. astr. Soc.*, **161**, 47 (1973).
- ¹⁶ Tsytovich, V. N., *Nonlinear Effects in Plasma* (Plenum, New York, 1970).
- ¹⁷ Branson, N. J. B. A., Elsmore, B., Pooley, G. G., and Ryle, M., *Mon. Not. R. astr. Soc.*, **156**, 377 (1972).
- ¹⁸ Mitton, S., *Astrophys. Lett.*, **13**, 19 (1973).
- ¹⁹ Mitton, S., and Mitton, J., *Mon. Not. R. astr. Soc.*, **158**, 245 (1972).

LETTERS TO NATURE

PHYSICAL SCIENCES

Apparent Loss of Angular Momentum in the Earth-Moon System

PANNELLA^{1,2} and others^{3,4} have presented palaeontological counts of the number of days per solar year (N) and per synodic month (n) during geologic time. This method is based on the cyclic growth increments of various fossils and has been confirmed in their modern counterparts^{1,5}. The observations extend back to 1.7×10^9 yr with some incomplete data to 2.8×10^9 yr.

In contrast, equivalent astronomical observations go back only some three centuries and, as Pannella¹ points out, the late capture theory of the Moon which stems from these measurements requires revision in the light of the palaeontological results. Fairbridge⁶ also argues against any catastrophic Earth-Moon interaction during the past 3.7×10^9 yr on the basis of biological continuity.

Following Runcorn⁷ we express the conservation of angular momentum L of the Earth-Moon-Sun system as

$$(1 + \beta)m(GM)^{2/3}(w^{-1/3} - w_0^{-1/3}) + (I\Omega - I_0\Omega_0) = 0 \quad (1)$$

where I is the Earth's rotational moment of inertia, m and M are the masses of Moon and Earth, respectively, and G is the gravitational constant. The sidereal spin of the Earth is Ω and the sidereal orbital angular velocity of the Moon w .

Subscript zero refers to the present time and β is the ratio of solar to lunar tidal couples. We are neglecting the small effect of rotation about the centre of mass of the Earth-Moon.

We can then write

$$\begin{aligned} \Omega &= w_s(N+1) \\ w &= w_s(N/n+1) \end{aligned} \quad (2)$$

where w_s is the sidereal angular velocity of the Earth about the Sun. If N_0 and n_0 correspond to the present time then a change in L given by N and n at another time will be

$$\begin{aligned} \Delta L &= Iw_s(N - N_0) + (1 + \beta)m(GM)^{2/3}w_s^{-1/3}[(N/n+1)^{-1/3} - \\ &\quad (N_0/n_0+1)^{-1/3}] \\ &= \Delta L_E + (1 + \beta)\Delta L_M \end{aligned} \quad (3)$$

where ΔL_E is the change in spin angular momentum of the Earth and ΔL_M is the corresponding change in lunar orbital angular momentum. Here we are assuming that w_s , G and I are constant. We discuss these factors later.

The reported pairs (N , n) are plotted in Fig. 1. These are mean values and they have been corrected upwards by about 1% on the basis of counts on recent animals². We note that n decreases with advancing time.

For comparison with these data, we have also plotted equation (3) for several values of $\Delta L/L_0$, with $\beta=0$. The curve for $\Delta L/L_0=0$ is of particular interest because it expresses the conservation of angular momentum of the Earth-Moon-Sun system under the assumptions mentioned. It has the remarkable characteristic that n reaches a maximum value of about

31.4 when $N \approx 550$. In fact, the data seem to depart increasingly from this limit as we go back in time. Consistent with the Precambrian point at 1.7×10^9 yr, Pannella⁸ has reported a measurement at 2.8×10^9 yr of $n \approx 45$ d which is not plotted.

Thus it appears from Fig. 1 that there is a continual loss of momentum from the oldest point of 1.7×10^9 yr (a Precambrian stromatolite) to the present. As indicated by the curves for $\Delta L/L_0 = 0.025$ and 0.05 , it amounts to a loss in L of some 5%. Estimates of β range from $1/3.4$ (ref. 9) to $1/11$ (ref. 10). Substitution of these values in equation (3) does not change the curves significantly and so we have neglected this factor.

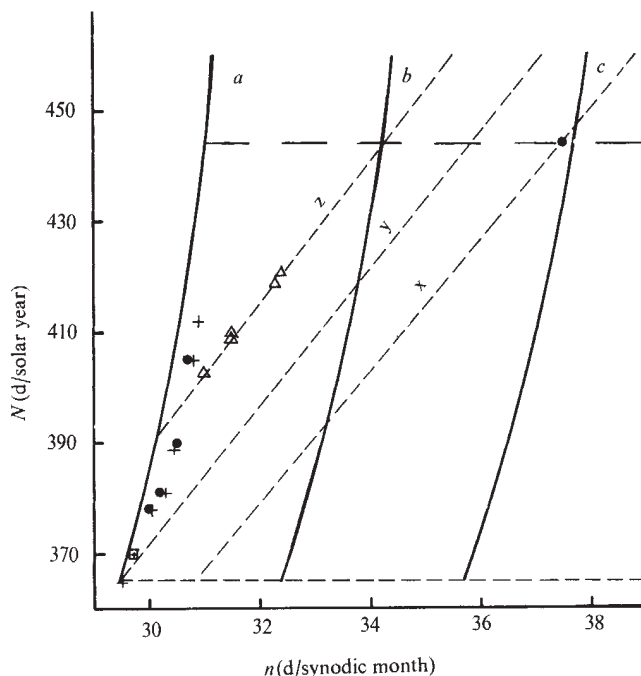


Fig. 1 The number of days per solar year as a function of the number of days per synodic month from counts of growth increments in fossil life forms, ranging in age from recent to Precambrian (1.75 aeons). ●, Pannella¹; △, Mazzullo³; □, Berry and Barker⁴; +, Pannella *et al.*². —: a, $\Delta L/L_0 = 0$; b, 0.025 ; c, 0.05 ; x, $\Delta L_M/L_0 = +0.011$; y, 0 ; z, -0.011 . — — —: top, $\Delta L_E/L_0 = 0.036$; bottom, 0 .

To aid in separating ΔL_E and ΔL_M we have also drawn in Fig. 1 straight lines corresponding to $\Delta L_E/L_0 = 0$ and 0.036 and $\Delta L_M/L_0 = 0$ and ± 0.011 . The Moon has experienced three distinct phases. From the Precambrian ($n = 37.5$) to the Silurian ($n = 32.4$) momentum was seemingly lost. This was followed by a period of constant momentum from Silurian to Pennsylvanian ($n = 30.4$) and finally there has been a gain of momentum from Pennsylvanian to the present. The first two phases are anomalous because tidal action can only serve to increase the lunar momentum. We note also that the Earth has undergone a loss in momentum over the same time interval and of magnitude considerably greater than that of the Moon. Can this behaviour be accounted for by changes in w_s , G or I ?

First, we consider w_s . Hipkin¹¹ has discussed the length of the sidereal year and concludes, on the basis of known influences, that it has been sensibly constant at least over the past aeon.

Second, Morrison¹² has deduced from ancient and modern lunar occultations of the stars that $\dot{G}$ is essentially zero. In any event $\dot{G}/G$ would be negative according to Dirac's¹³ ideas and of the order of 10^{-10} yr^{-1} (ref. 14). A negative value of $\dot{G}$ would serve only to increase the discrepancies.

With this in mind, we examine the effect of a change in I assuming L is constant. Runcorn⁷ has already considered the

interval from the Devonian to the present and has found $\Delta I/I_0 \sim 0$ during that time. Using Runcorn's approach, for the past 1.7×10^9 yr we find that $\Delta I/I_0$ ranges from 0.25 to 0.30 for values of β from $1/3.4$ to 0 . That is, if angular momentum has been conserved, then I has apparently increased by about 25 to 30% during that interval of time, with most of the increase occurring before the Devonian.

Runcorn^{7,15,16} has given a comprehensive review of theories of the Earth's evolution which involve a change in I . Several theories¹⁷⁻¹⁹ lead to a decrease in I with time and they would only increase the apparent loss of angular momentum. On the other hand, Egyed²⁰ and Creer²¹ have suggested that the Earth is expanding at a rate of 0.5 to 1.2 mm yr^{-1} which could possibly increase I . But as Creer¹⁴ points out, "the evidence for expansion is somewhat indecisive and it cannot be accounted for theoretically". In addition, concurrent or subsequent core formation should tend to negate the effects of expansion.

The angular momentum of the Earth is certainly influenced by changes in I . On the other hand, the anomalous behaviour of the lunar momentum from Precambrian to Pennsylvanian cannot be accounted for by any of the means discussed above, particularly by a change in I . Cosmological effects may, however, play a part, and we will discuss these elsewhere.

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- ¹ Pannella, G., *Astrophys. Space Sci.*, **16**, 212 (1972).
- ² Pannella, G., MacClintock, C., and Thompson, M. N., *Science*, **162**, 792 (1968).
- ³ Mazzullo, S. J., *Geol. Soc. Amer. Bull.*, **82**, 1085 (1971).
- ⁴ Berry, W. B. N., and Barker, R. M., *Nature*, **217**, 938 (1968).
- ⁵ Clarke, G. R., *Science*, **161**, 800 (1968).
- ⁶ Fairbridge, R. W., *Ann. NY Acad. Sci.*, **187**, 88 (1972).
- ⁷ Runcorn, S. K., *Nature*, **204**, 823 (1964).
- ⁸ Anon., *New Scientist*, **56**, 439 (1972).
- ⁹ Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth*, 201 (Cambridge, London, 1960).
- ¹⁰ Newton, R. R., *Geophys. J. Roy. Astron. Soc.*, **14**, 505 (1968).
- ¹¹ Hipkin, R. G., in *Paleogeophysics* (edit. by Runcorn, S. K.), **54** (Academic, New York, 1970).
- ¹² Morrison, L. V., *Nature*, **241**, 519 (1973).
- ¹³ Dirac, P. A. M., *Proc. Roy. Soc. A*, **165**, 199 (1938).
- ¹⁴ Creer, K. M., in *International Dictionary of Geophysics* (edit. by Runcorn, S. K.), 388 (Pergamon, New York, 1967).
- ¹⁵ Runcorn, S. K., in *The Earth-Moon System* (edit. by Marsden, B. G., and Cameron, A. G. W.), 82 (Plenum, New York, 1966).
- ¹⁶ Runcorn, S. K., in *The Application of Modern Physics to the Earth and Planetary Interiors* (edit. by Runcorn, S. K.), 47 (Interscience, 1969).
- ¹⁷ Lyttleton, R. A., *Proc. Roy. Soc. A*, **275**, 1 (1963).
- ¹⁸ Urey, H. C., *The Planets* (Yale Univ., New Haven, 1952).
- ¹⁹ Runcorn, S. K., *Phil. Trans. Roy. Soc. A*, **258**, 228 (1965).
- ²⁰ Egyed, L., *Nature*, **178**, 534 (1956).
- ²¹ Creer, K. M., *Nature*, **205**, 539 (1965).

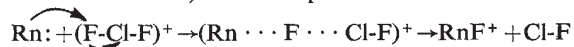
Oxidant for Trapping Atmospheric Radiokrypton

RECENTLY, Stein¹ presented a chemical method of removing radioactive xenon from the effluent gases of nuclear power plants and fuel reprocessing plants. His chemical oxidant, $\text{O}_2 + \text{SbF}_6^-$, successfully trapped xenon and radon, but not

krypton. This result is reasonable if the initial reaction is electron transfer from the noble gas, Ng, to the O_2^+ cation. Multistep fluorine (F and F^-) transfer then produces NgF^+ . More quantitatively, the experimental ionization potentials for Rn and Xe, 10.748 eV and 12.130 eV (ref. 2) are, respectively, less than and commensurate with that of O_2 , 12.07 eV (ref. 3). Accordingly, electron transfer may be expected. Because the ionization potential of Kr is² 13.999 eV, however, no reaction is expected between O_2^+ and Kr.

Stein earlier showed^{4,5} that ClF_2^+ , BrF_2^+ and IF_4^{3+} salts react with radon to produce RnF^+ salts. These radon salts may be formed by electron transfer from the radon to the fluoro-halogen cations and subsequent fluorine transfer analogous to the above mechanism for O_2^+ .

Alternatively, one may hypothesize an S_N2 displacement reaction on fluorine, for example:



At this time, there is insufficient evidence to prove or disqualify either mechanism although the latter seems more plausible^{6,7}. The assumption that this is an S_N2 reaction suggests other reagents for testing for reactions with Xe, Rn, and Kr. That is, there must be some Lewis base, Lb, such as $LbF^+ + Ng \rightarrow NgF^+ + Lb$ is exothermic. Because SbF_6^- is the logical anion because of its high oxidative stability, it is required in addition that $Lb + SbF_6^- \rightarrow LbSbF_6 + F^-$ of possible reagents.

Here I consider only ΔH and not ΔG . Ionization potentials are in eV and heats of formation and reduction kcalorie mol^{-1} (1 eV = 23.0609 kcalorie mol^{-1}). I neglect the entropy term because at the desired conditions (near ambience) the magnitude of this correction is much smaller than the uncertainty in many molecular thermodynamic values. Further, I only consider the energetics of the cation. Madelung crystal energy may be safely neglected because the size of the desired Lewis base is almost definitely larger than the noble gases⁸. As such, crystal stabilization is anticipated to increase on reaction although SbF_6^- is such a large anion that this effect is probably small.

I first compute $\Delta H_f^\circ(g)$ for KrF^+ , XeF^+ and RnF^+ . The first two ions have almost equal dissociation energies; $D_0(KrF^+) \geq 1.58$ eV = 36 kcalorie mol^{-1} and $D_0(XeF^+) = 2.03$ eV = 47 kcalorie mol^{-1} (ref. 9). Very accurate quantum chemical calculations have been made for the first ion¹⁰ which give $D_0(KrF^+) = 1.90$ eV, but corresponding studies are lacking on XeF^+ and RnF^+ . From the experimental heats of formation of F^+ , Kr^{3+} , Xe^{3+} and Rn^{2+} , the experimental value for $D_0(XeF^+)$ and the calculated value for $D_0(KrF^+)$ I obtain the following heats of formation: KrF^+ , 298 kcalorie mol^{-1} ; XeF^+ , 252 kcalorie mol^{-1} ; RnF^+ , ≤ 263 kcalorie mol^{-1} (obtained by assuming that RnF^+ is unbound). If all three NgF^+ have essentially the same dissociation energy, the value for RnF^+ is 216 kcalorie mol^{-1} .

In order that $LbF^+ + Ng \rightarrow NgF^+ + Lb$ be exothermic, it is necessary that $D_0(Lb-F) + IP(Ng) < D_0(Ng-F) + IP(Lb)$. Accordingly, I require Lb to have a high ionization potential and a weak bond to fluorine. A particularly simple selection for Lb is N_2 which has an ionization potential of 15.57 eV (ref. 3). The $N_2^+ - F$ bond strength, $D_0(N_2^+ - F)$, has not been directly measured in N_2F^+ salts^{11,12} but may be computed from $-\Delta H_f^\circ(N_2F^+) + \Delta H_f^\circ(N_2^+) + \Delta H_f^\circ(F) = -321 + 359 + 19 = 57$ kcalorie mol^{-1} using the experimental values of ref. 3. Using this value for $D_0(N_2^+ - F)$ gives the following heats of reaction for NgF^+ synthesis: KrF^+ , 23 kcalorie mol^{-1} ; XeF^+ , 69 kcalorie mol^{-1} ; RnF^+ , ≥ 48 kcalorie mol^{-1} . (Even with the experimental lower bound for $D_0(KrF^+)$, the synthesis of KrF^+ would be exothermic by 16 kcalorie mol^{-1} .) As such, $N_2F^+ SbF_6^-$ seems to be a simple reagent for removing radioactive Kr, Xe and Rn from the atmosphere. With the exception of KrF^+ , the exothermicity of the reaction is sufficient to break the newly formed NgF^+ bond. I cannot predict quantitatively how often the Xe^+ or Rn^+ will recombine with the same F atom to form NgF^+ by the cage effect¹³. But because of the

mobility of free atoms, I predict that XeF^+ and RnF^+ formed by this mechanism will be relatively rare. Stein's evidence¹ strongly suggests that Xe^+ and Rn^+ ions formed *in situ* can form XeF^+ and RnF^+ . But I expect few of these noble gas atoms to remain in the absorbate trap bed. When recombination does not occur, the F_2 is much more environmentally undesirable than the O_2 in Stein's procedure. As such, after treating the effluent nuclear reactor or reprocessed fuel gases with $O_2 + SbF_6^-$ and then with $N_2F^+ SbF_6^-$ to remove sequentially the xenon (and radon) and krypton, a final treatment with a reducing agent such as iron filings to remove whatever F_2 is formed is probably advisable.

The N_2F^+ salts slowly hydrolyse to form N_2O and HF (ref. 12). But little complication should arise in using this procedure as a radiokrypton trap because removal of atmospheric moisture is customary before treatment of the radioactive air with chemical oxidants^{4,5}. In principle, the N_2F^+ cation could react with O_2 to form $N_2 + F + O_2^+$ because this reaction is 14 kcalorie mol^{-1} exothermic. But N_2F^+ salts seem to be stable in dry air^{11,12}. Even should the reaction occur, the O_2^+ formed will remove Xe and Rn unscavenged by the first step of treatment and the reducing agent should remove whatever fluorine is formed here.

I note that $N_2F_3^{12+}$ should also transfer F^+ to Kr, Xe and Rn. Because the heats of formation of $N_2F_3^+$ and *trans* and *cis*- N_2F_2 are respectively 339, 20 and 17 kcalorie mol^{-1} (ref 3). $\Delta H_f^\circ(N_2F^+) - \Delta H_f^\circ(N_2)$ is almost identical to $\Delta H_f^\circ(N_2F_3^+) - \Delta H_f^\circ(N_2F_2)$. Therefore all of the energetics conclusions for N_2F^+ are applicable here. Both isomers of N_2F_2 are thermodynamically unstable with respect to decomposition to form $N_2 + F_2$. The heat of formation⁸ of KrF_2 is 14 kcalorie mol^{-1} , of XeF_2 is -28 kcalorie mol^{-1} and of RnF_2 is clearly negative. In all cases the reaction of N_2F_2 with Ng to form $NgF_2 + N_2$ is exothermic. But reaction between such non-polar species is expected to be slow. Because both isomers of N_2F_2 boil at less than $-100^\circ C$ (ref. 14) it does not seem to be feasible to capitalize on the reaction: $2Ng + N_2F_3^+ \rightarrow NgF_2 + NgF^+$ (or $Ng_2F_3^+$ as in the case of Xe (ref. 15)) + N_2 , that is $Ng + N_2F_3^+ \rightarrow NgF^+ + N_2F_2$, $N_2F_2 + Ng \rightarrow NgF_2 + N_2$ in my reaction system.

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¹ Stein, L., *Nature*, **243**, 30 (1973).

² Moore, C. E., *Nat. Stand. Ref. Data Ser.*, 34 (US National Bureau of Standards, 1970).

³ Franklin, J. L., Dillard, J. G., Rosenstock, H. M., Herron, J. T., Draxl, K., and Field, F. H., *Nat. Stand. Ref. Data Ser.*, 26 (US National Bureau of Standards, 1969).

⁴ Stein, L., *Science*, **168**, 362 (1970).

⁵ Stein, L., *Science*, **175**, 1463 (1972).

⁶ Lustig, M., Pitochelli, A. T., and Ruff, J. K., *J. Amer. Chem. Soc.*, **84**, 2841 (1962).

⁷ McKee, D. E., Adams, C. J., Zalkin, A., and Bartlett, N., *J. Chem. Soc. Chem. Commun.*, 26 (1973).

⁸ Huheey, J. E., *J. Chem. Ed.*, **45**, 791 (1968).

⁹ Berkowitz, J., and Chupka, W. A., *Chem. Phys. Lett.*, **7**, 447 (1970).

¹⁰ Liu, B., and Schaeffer III, H. F., *J. Chem. Phys.*, **55**, 2369 (1971).

¹¹ Moy, D., and Young, A. R., *J. Amer. Chem. Soc.*, **87**, 1889 (1965).

¹² Qureshi, A. M., and Aubke, F., *Canad. J. Chem.*, **48**, 3117 (1970).

¹³ Kosower, E. M., *An Introduction to Physical Organic Chemistry*, 352 (Wiley, New York, 1968).

¹⁴ Cotton, F. A., and Wilkinson, G., *Advanced Inorganic Chemistry*, third ed., 564 (Interscience, New York, 1972).

¹⁵ Bartlett, N., *et al.*, *Chem. Commun.*, 1048 (1968).

A Fossil Triple Junction in the NE Atlantic West of Biscay

MARINE magnetic data in the Bay of Biscay¹ reveal a fan shaped pattern of linear magnetic anomalies radiating from the SE corner of the Bay of Biscay. The trend direction of the lineations in southern Biscay is E-W, parallel to the northern Iberian continental margin, and it becomes NW-SE towards the French continental margin. An aeromagnetic survey², with a regular network of 10 km space lines—considerably more than were available for the marine survey—confirmed the results of the marine work by revealing the same radiating pattern of magnetic anomalies. Matthews and Williams's lineations¹ are shown superimposed over the aeromagnetic anomaly contours in Fig. 1 to illustrate the similarity between the two interpretations. The similarity appears although different regional magnetic gradients have been subtracted from the two data sets. The aeromagnetic anomalies are relative to the IGRF³, and the Bullard *et al.*⁴ regional gradient has been subtracted from the earlier marine work.

Because the aeromagnetic survey extends west only to 10° W, two N-S tracks were run on board MV Researcher in 1971 and RRS Shackleton in 1972 at 12½° W and 13½° W, respectively, to establish the westwards continuity of the Biscay lineations out into the NE Atlantic.

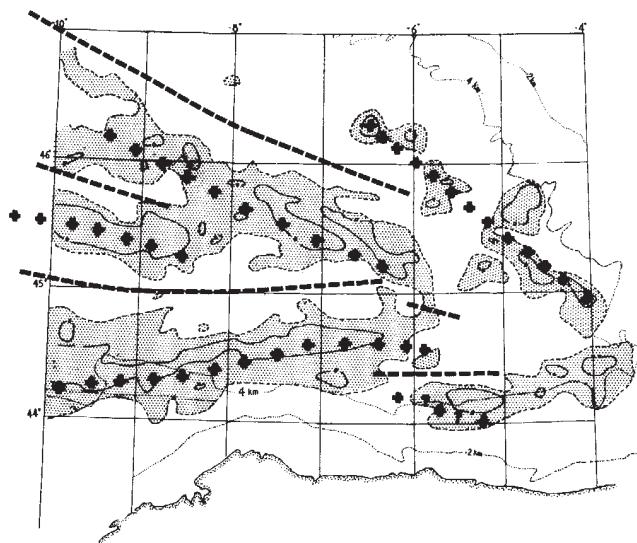


Fig. 1 Magnetic lineations in the Bay of Biscay. The stippled areas mark areas of $> -100 \gamma$ from the aeromagnetic contours². The positive and negative symbols indicate lineations from the marine magnetic interpretation¹.

The magnetic lineations in the NE Atlantic have been determined by Williams and McKenzie⁵, who remark on their westward curvature which is particularly marked in the older lineations, anomalies 32 (75 m.y.) to 24 (60 m.y.), and on the change in anomaly trend which occurs at approximately 45° N. They show that the NE Atlantic lineation 31-32 can be continuously traced between latitudes 52° N and 37° N except for a discontinuity of approximately 60 km near 45° N, where the profile character of this normally distinctive anomaly is lost (Fig. 2).

The magnetic data used by Williams and McKenzie have been contoured (Fig. 3) and in this form demonstrate more clearly the NNW-SSE anomaly trend in the Atlantic north of Biscay, changing to NNE-SSW south of approximately 45° N. The figure also shows the radiating pattern of magnetic lineations as depicted by the aeromagnetic contours east of 10° W in the Bay of Biscay and their continuation west into the Atlantic. The IGRF does not fit well across the Bay of Biscay area and tends to produce a predominance of negative anomalies. I have

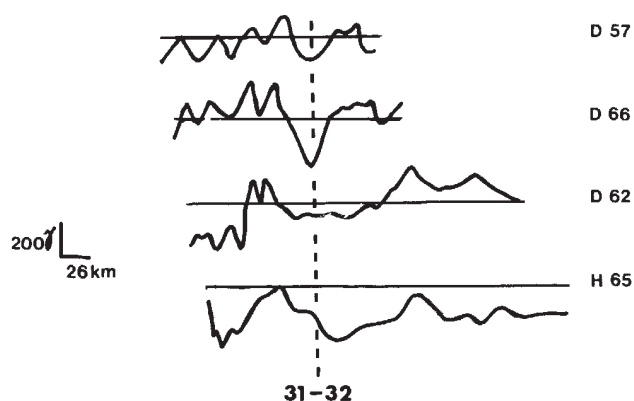


Fig. 2 Magnetic anomaly profiles crossing anomaly 31-32, from RRS Discovery 1957, 1966 and 1962 and CSS Hudson 1965. The two upper profiles show the normal character of anomaly 31-32, and the lower two profiles indicate its change in profile near 45° N. The four profiles have been projected onto an E-W plane and, from upper to lower, cross anomaly 31-32 lineation at 46½°, 43½°, 45½° and 45° N respectively.

therefore used the 100 γ contour to delineate the trends of the anomalies in this region.

The Earth's magnetic field was reversed during the period of formation of anomaly 31-32. This anomaly is shown in Fig. 3 by the 100 γ contour near 45° N, 15° W where it bends through about 150°, from NNW-SSE to NNE-SSW, and also has an E-W trending limb which forms part of the Bay of Biscay pattern of radiating lineations. This relationship of linear anomalies suggests that a ridge-ridge-ridge triple junction⁶ existed at the time of anomaly 31-32 (73 m.y. ago) to the west of the Bay of Biscay at approximately 45½° N, 15° W, where the Mid-Atlantic Ridge was connected to a now-extinct mid-ocean ridge in the Bay of Biscay. The trend of the eastern branch of anomaly 31-32 suggests that the Biscay ridge had an E-W orientation and that the spreading motion from it caused the formation of the Bay of Biscay with the associated anticlockwise rotation of Iberia relative to Eurasia. Thus a three-plate geometry existed during this time between the North American, Eurasian and Iberian plates. The eastern limit of the Iberian-Eurasian plate boundary is not known and it may have terminated in the Tethyan Ocean, which then existed to the west of Iberia.

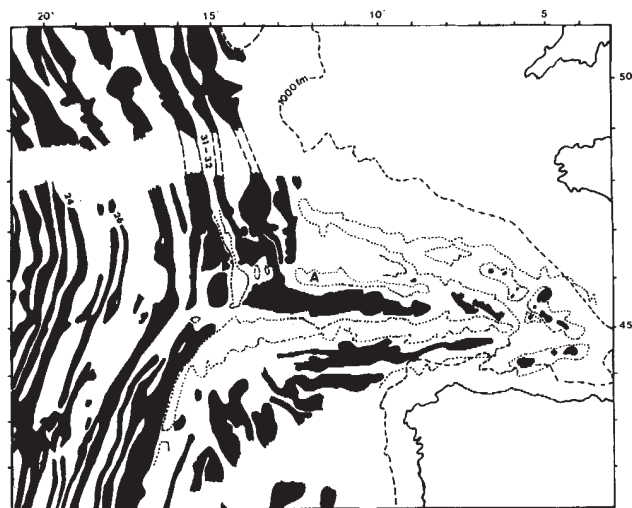


Fig. 3 Magnetic anomaly contours relative to the IGRF in the NE Atlantic and Bay of Biscay. Positive anomalies are shaded black. The dotted line in and around the Bay of Biscay is the -100γ contour. The contours are drawn from marine data except east of 10° W, where the aeromagnetic contours are shown. The lineation numbers are conventionally numbered^{1,5} as determined by ref. 5.

Anomaly 32 has been identified in the Atlantic north of Biscay⁶ and it seems to be continuous with an E-W positive anomaly in Biscay lying to the north of anomaly 31-32. To the south of the triple junction the available data do not yet allow the inferred bend in anomaly 32 to be mapped. Anomaly 31-32 is the youngest North Atlantic lineation which shows any connexion with the Biscay lineations and it thus appears to be the youngest anomaly in the Bay of Biscay. This implies a Mesozoic episode of seafloor spreading in the Bay of Biscay, terminating during the formation of anomaly 31-32 approximately 73 m.y. ago.

Various different axes of symmetry for the Biscay magnetic lineations have been suggested; Le Pichon⁷ observed symmetry about a central positive lineation, and work by Schouten *et al.*⁸

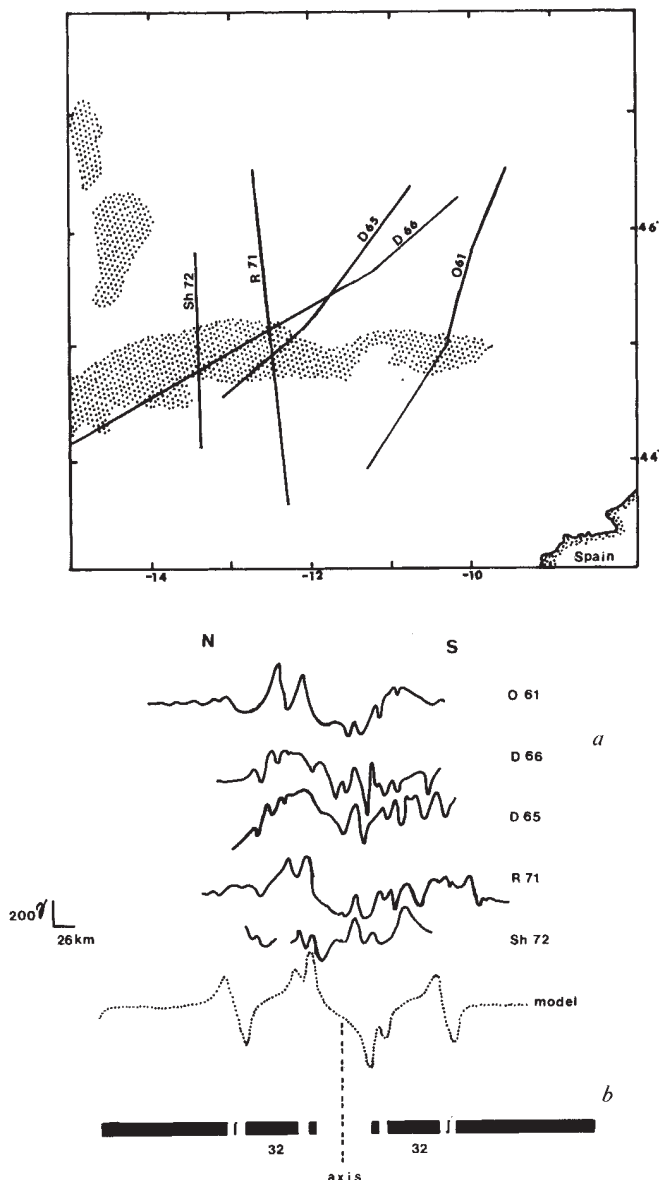


Fig. 4 *a*, Five magnetic anomaly profiles across western Biscay made aboard HMS Owen 1961, RRS Discovery 1965 and 1966, MV Researcher 1971 and RRS Shackleton 1972. The profiles have been projected onto a N-S plane. The stippled area indicates the parts of anomaly 31-32 which are $< -100 \gamma$. *b*, A calculated anomaly profile across a N-S symmetric pattern of magnetized blocks. Normally magnetized blocks are shaded. Their configuration represents the end of the Heirtzler *et al.* geomagnetic reversals time scale¹⁶ as modified by McKenzie *et al.* and Sclater¹⁷. The profile was calculated with a palaeolatitude of 30°N and at a spreading rate of 3.7 cm yr^{-1} , and is aligned with the observed profiles for comparison.

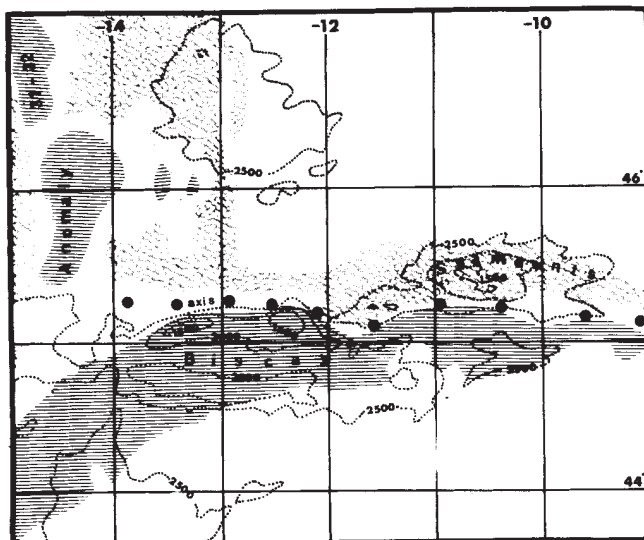


Fig. 5 The relationship between the Biscay Seamounts and the magnetic lineations. Bathymetric contours in fathoms are superimposed over areas of positive magnetic anomaly (wavy shading) and areas of $< -100 \gamma$ (horizontal shading). The axis of the proposed Biscay ridge is shown by filled circles.

on magnetic anomaly profiles across the western end of the Bay of Biscay has indicated a symmetry about a negative anomaly at approximately 46°N (anomaly A in Fig. 3).

Model calculations demonstrate that an N-S direction of spreading from an E-W oriented ridge-crest will not produce a symmetric pattern of anomalies, since the peaks and troughs will be displaced to the southern ends of the normally and reversely magnetized blocks respectively (Fig. 4*b*). Thus the axis of the former Biscay mid-ocean ridge will not lie along any positive or negative anomaly lineation, but between the two. It also follows that the anomaly lineations produced by a symmetrical pattern of magnetized blocks will produce an asymmetric pattern of lineations as seen on a map. Thus, according to the triple-junction hypothesis, the Biscay ridge axis lay immediately to the north of the E-W trending part of anomaly 31-32. The axis follows the E-W col lying between the two separate masses of the Biscay Seamounts (Fig. 5), suggesting that these seamounts represent relicts of a former mid-ocean ridge crest, which for some reason has not subsided to the depth of the remainder of the Bay of Biscay seafloor. The proposed axis does not lie symmetrically within the area of oceanic crust suggested by Bacon⁹ on seismic refraction evidence, and which may also be defined on the aeromagnetic map⁹ by the sharp boundary between the anomalous and the magnetically quiet areas of the Bay of Biscay.

If this hypothesis is correct and the axis of symmetry is as indicated by the model in Fig. 4*b*, then one should see correlations between different magnetic profiles crossing the Bay of Biscay. The five westernmost profiles (Fig. 4*a*) have been aligned about the axis of symmetry suggested by the triple junction hypothesis and show possible correlations. The double peak character of the anomaly to the north of the axis is characteristic and can be traced through the Bay of Biscay magnetic profiles to 6°W . The other correlations are possibly not entirely convincing and further survey tracks are planned in the vicinity of the triple junction later this year. There is evidence¹⁰ that this area has suffered from tectonic movements during the Eocene and again in the Miocene during the principal Alpine orogeny. That activity may have confused the magnetic anomalies in such a way that they now lie at the effective limits of the anomaly correlation technique of analysis. Those tectonic movements may also be associated with a clockwise rotation of Iberia relative to France¹¹ which could also explain the asymmetric distribution of oceanic crust about the

proposed ridge axis, if part of the Biscay seafloor has been consumed by the North Spanish Trough.

The proposed triple junction is integrally linked with theories of the evolution of the Bay of Biscay. The formation of the Bay is not yet understood, although a variety of contradictory hypotheses of its origin have been put forward. Of these hypotheses only those of Matthews and Williams¹ and Le Pichon *et al.*¹² are concerned with the origin of the magnetic anomalies and neither of these is consistent with the conception of a triple junction put forward here. The suggested Mesozoic rotation of Iberia by 10° to 20° about a pole in Corsica¹³ is consistent with the proposed triple junction, although the angle of rotation is less than the 35° indicated from palaeomagnetic evidence¹⁴. None of these hypotheses are widely accepted, but if the existence of the triple junction is substantiated by further surveying it will add another constraint upon future theories of the evolution of the Bay of Biscay.

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- ¹ Matthews, D. H., and Williams, C. A., *Earth Planet. Sci. Lett.*, **4**, 315 (1968).
- ² Le Borgne, E., Le Mouel, J.-L., and Le Pichon, X., *Earth Planet. Sci. Lett.*, **12**, 287 (1971).
- ³ Cain, J. C., Hendricks, S., Daniels, W. E., and Jensen, J. C., *Data Users' Note NSSDC 68-11* (NASA, 1968).
- ⁴ Bullard, E. C., Hill, M. N., and Mason, C. S., *Geomagnetica*, **185** (1962).
- ⁵ Williams, C. A., and McKenzie, D., *Nature*, **232**, 168 (1971).
- ⁶ McKenzie, D. P., and Morgan, W. J., *Nature*, **224**, 125 (1969).
- ⁷ Le Pichon, X., *Earth Planet. Sci. Lett.*, **5**, 251 (1969).
- ⁸ Schouten, J. A., Collette, B. J., and Rutten, K. W., *Symp. sur l'Histoire Structurale du Golfe de Gascogne* (Editions Technip, 1971).
- ⁹ Bacon, M., thesis, Cambridge Univ. (1970).
- ¹⁰ Anonymous, *Symp. sur l'Histoire Structurale du Golfe de Gascogne* (Editions Technip, 1971).
- ¹¹ Le Pichon, X., and Sibuet, J.-C., *Earth Planet. Sci. Lett.*, **12**, 83 (1971).

- ¹² Le Pichon, X., Bonnin, J., Francheteau, J., and Sibuet, J.-C., *Symp. sur l'Histoire Structurale du Golfe de Gascogne* (Editions Technip, 1971).
- ¹³ Montadert, L., and Winnock, E., *Symp. sur l'Histoire Structurale du Golfe de Gascogne* (Editions Technip, 1971).
- ¹⁴ Van der Voo, R., and Zijdeveld, J. D. A., *J. Geophys. Res.*, **76**, 3913 (1971).
- ¹⁵ Pitman, W. C., and Heirtzler, J. R., *Science*, **154**, 1164 (1966).
- ¹⁶ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).
- ¹⁷ McKenzie, D. P., and Sclater, J. G., *Geophys. J. Roy. Astron. Soc.*, **25**, 437 (1971).

Vertical Structure of Semi-diurnal Tidal Currents in the Bay of Biscay

IN the winter of 1970–71 the National Institute of Oceanography deployed four current meter moorings on the continental slope in the north of the Bay of Biscay near 47° 30' N 08° 30' W (Fig. 1). Here we report data from two of these moorings, with serial numbers 072 (47° 39.6' N 07° 14.2' W) and 074 (47° 31.4' N 08° 27.9' W). One (072) was on the continental shelf in 190 m of water with a single record (07201) at 54 m. The other had a total of six records (07401–6) at depths of 91, 292, 492, 990, 1,487 and 1,986 m, the lowest record being 15 m above the sea bed. The data were obtained with Aanderaa¹ current meters and both the moorings had subsurface buoyancy to decouple the mooring lines from surface wave activity. The records were all of duration approximately 20 d, that on 072 being from February 1 to February 22, 1971, and those on mooring 074 lasting from February 5 to February 26. Current speed and direction were measured every 5 min in each record.

We have used density stratification of the water column in the vicinity of the current meter moorings indicated by STD profiles taken on RRS Discovery station 7520. This station consisted of a series of 25 2-h profiles of temperature and salinity to a depth of 2,000 m starting at 1030Z on February 7,

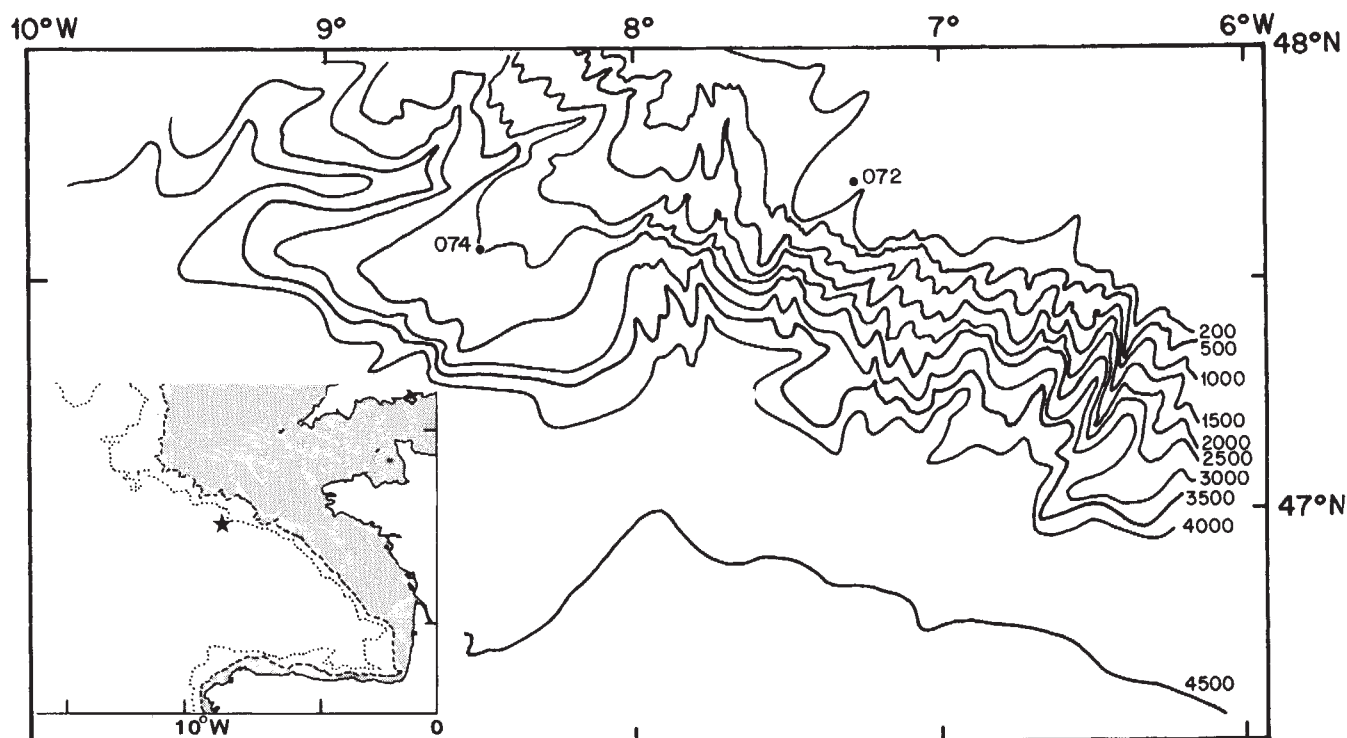


Fig. 1 Topography (contours in m) in the north Biscay area and positions of current meter moorings. Insert, Bay of Biscay showing region of study (★).

1971. All the profiles were made within 3.6 km of 47° 29.0' N 08° 33.0' W (about 7 km from mooring 074). From this series we obtained a smoothed profile of the Brunt-Väisälä stability frequency $N = \sqrt{(g/\bar{\rho})(d\rho/dz)}$ (where $\bar{\rho}$ is the mean density and $d\rho/dz$ is the vertical $\bar{\rho}$ density gradient), which hopefully is representative of the average stratification of the water column during the experiment (Fig. 2).

The technique used has been described in detail elsewhere^{2,3}.

The data from the current meter time series were Fourier analysed using a fast Fourier transform⁴ to obtain the Fourier coefficients for frequencies approximating closely to those of the solar and lunar semi-diurnal tidal components (S_2 , 12.0000 h; M_2 , 12.4206 h). This was achieved by using a data series 360 h long which resulted in adjacent estimates at 12.0000 h and 12.4138 h. The total data length for each of the six levels of mooring 074 was some 500 h and Fourier analysis was performed in two 360 h lengths, the first starting at the beginning of the time period common to all records (1550Z February 5, 1971) and the second ending at the end of the common time period (starting 0815Z February 11). These pieces overlapped by 220 h, about 60% of the individual lengths. The single record from mooring 072 on the continental shelf started and finished earlier than the records from mooring 074. Only the first time period is common to this mooring and to 074, and the record for that time period was subjected to spectral analysis.

From the data on the stratification of the water column we computed the internal wave modes for M_2 and S_2 . These modes are solutions for the vertical velocity W of $(\omega^2 - f^2)d/dz(\rho\delta W/\delta z) - \rho(\omega^2 - N^2)k^2 W = 0$ where f is the Coriolis

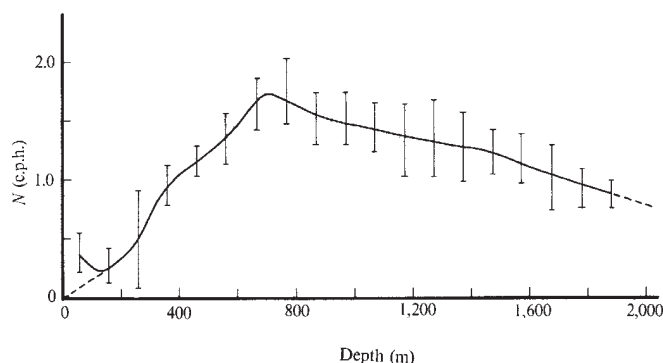


Fig. 2 Vertical profile of Brunt-Väisälä frequency (N) computed for density differences over a depth interval of 100 m. The computations require $N=0$ at the surface (---). Bars show the spread of observed values at various levels.

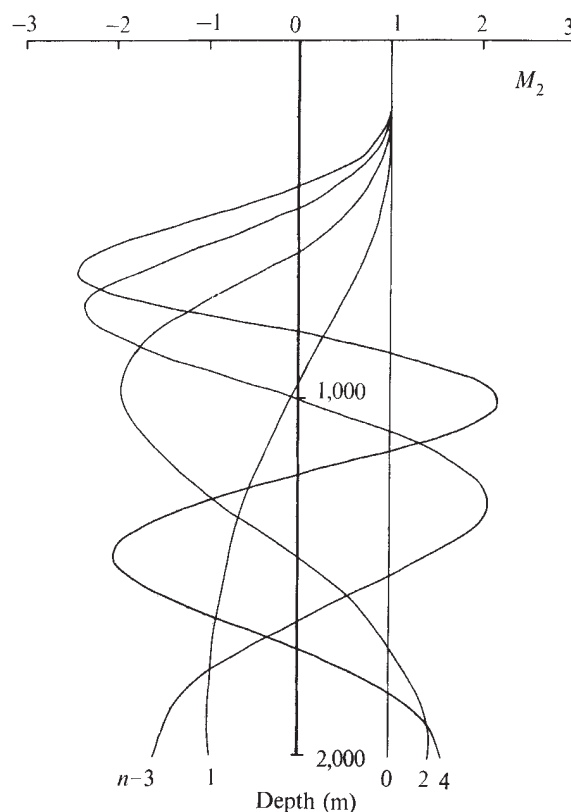


Fig. 3 The horizontal velocity structure for the barotropic and four baroclinic modes for the M_2 frequency. Values are normalized to unity at the sea surface. (The structure for the S_2 frequency is almost identical.)

parameter; N is the Brunt-Väisälä frequency; ρ is the density; and k^2 is the eigenvalue (the square of the horizontal wave-number).

The solution is obtained subject to the boundary conditions

$$W=0 \text{ at the sea bed (assumed to be flat)}$$

$$(\omega^2 - f^2)(\delta W/\delta z) - gk^2 W = 0 \text{ at the free upper surface}$$

We assumed the horizontal velocity to be proportional to the z -derivative of the vertical velocity and all horizontal velocity values were normalized to be unity at the sea surface (Fig. 3).

Table 1 Dimensions, Orientations, Phases and Rotation Senses for the M_2 and S_2 Tidal Ellipses and for the Observations of Cartwright and Woods⁵

Record	Depth (m)	Major axis cm s^{-1}	Minor axis cm s^{-1}	Orientation of major axis $^\circ\text{T}$	Phase h min	Sense	Major axis cm s^{-1}	Minor axis cm s^{-1}	Orientation of major axis $^\circ\text{T}$	Phase h min	Sense
07401	91	7.09	1.50	175	-2 31	C	7.29	4.82	15	+5 41	C
		6.06	0.06	173	-2 30	A	7.33	5.12	12	+5 59	C
07402	292	5.90	1.15	160	-1 46	C	5.83	4.60	177	+0 22	C
		3.73	0.96	154	-1 08	A	6.70	4.72	167	+0 49	C
07403	492	3.11	0.52	140	-1 47	A	6.60	4.40	142	-0 24	C
		5.57	1.01	155	-2 19	C	5.31	3.10	141	-0 49	C
07404	990	5.18	0.20	127	-0 56	C	6.93	2.75	107	+2 29	C
		6.81	1.97	115	-0 47	C	6.13	2.40	122	+2 15	C
07405	1,487	4.26	1.23	180	-3 09	C	2.82	0.32	67	+2 23	A
		6.20	0.25	24	+2 17	A	5.25	2.33	84	+1 37	C
07406	1,986	3.77	0.72	46	+0 47	A	6.56	3.17	59	+2 56	C
		4.16	1.70	7	+3 09	A	8.42	5.62	40	+2 51	C
07201	Shelf	37.29	25.28	25	+8 25	C	20.61	14.34	30	+4 06	C
Cartwright and Woods ⁵	41.25	29.75	27			C	15.25	10.25	27		C

The phase values refer to the times at which the current reaches its maximum value in the orientation direction to the passage of the Sun or Moon at the Greenwich meridian. No phases can be deduced from the Cartwright and Woods data.

The Fourier coefficients were then fitted by a set of the eigenfunctions using a least squares technique³. In this experiment data were available from six levels and estimates of the energies in the barotropic and first four baroclinic modes were made.

The results of the Fourier analysis of the data from both moorings are given in Table 1 in terms of the dimensions, orientations, senses of rotation and phases of the M_2 and S_2 tidal ellipses at the various levels on mooring 074 and at mooring 072 on the continental shelf.

The directions of the ellipse axes (Fig. 4) differ very little from the first data piece to the second for either frequency component but differences are greatest in the lower half of the water column.

The phase relationships between the currents and the crossing of the Greenwich meridian by the appropriate body (Moon or Sun) are also quite constant for the M_2 component at all but the deeper levels and for S_2 the phase relationships to the Sun are best defined in the surface layers.

There is a clear difference in the degree of polarization of the tidal ellipses for the two components, the ellipses for M_2 being in general "thin" (highly polarized) and those for the S_2 tide being less polarized. The high ellipticity of the M_2 tides probably accounts for the fact that there are several cases in which the sense of rotation of the ellipse changes between the two data pieces. Whenever a counter-clockwise rotation is computed the ellipse is small or "thin". So the errors in the ellipse dimensions may be greater than the dimensions of the minor axes and give rise to the change in rotation sense. Probably, the ellipses for both components and at all levels are described in a clockwise sense.

The current ellipses from the record from mooring 072 on the continental shelf have considerably greater dimensions than those in deeper water. Both tidal species have the ellipse major axes perpendicular to the general trend of the bottom

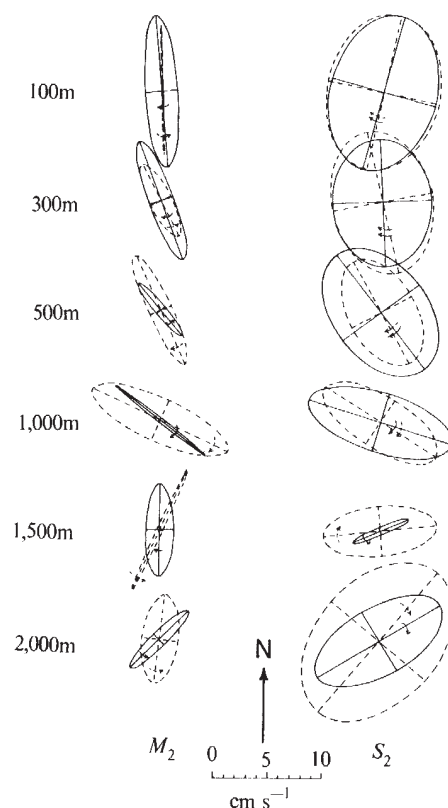


Fig. 4 Dimensions and senses of rotation of the M_2 and S_2 tidal ellipses from mooring 074 at the six observed levels. Solid lines refer to the first data piece (starts 1550Z February 5, 1971) and dashed lines to the second (starts 0815Z February 11).

Table 2 The Results of Fitting the Modal Structures deduced from the Density Profile to the Observed Data Series from Mooring 074

Mode No.	Modes fitted	Maximum cm s ⁻¹	Minimum cm s ⁻¹	Orientation °T S ₂ First piece	Phase h min Sense	Energy %	Maximum cm s ⁻¹	Minimum cm s ⁻¹	Orientation °T S ₂ Second piece	Phase h min Sense	Energy %	
0	0+4	2.44	0.38	122	+1 4 A		2.45	0.05	109	+1 19 C		
	0+3	2.12	0.75	109	+1 46 A		2.25	0.17	111	+1 37 A		
	0+2	2.05	0.81	111	+1 41 A		2.34	0.08	114	+1 36 A		
	0+1	1.53	1.18	153	+0 12 A	9.3	1.63	0.68	110	+1 31 A	8.1	
1	0+4	4.88	4.12	63	-3 17 C		5.02	4.44	59	-3 41 C		
	0+3	4.46	3.58	52	-3 47 C		5.08	4.19	45	-4 14 C		
	0+2	5.05	4.07	56	-3 47 C		5.68	4.68	38	-4 09 C		
	0+1	5.20	4.42	60	-3 44 C	68.6	5.67	4.73	42	-4 07 C	71.2	
2	0+4	2.23	1.75	124	-3 41 C		2.27	1.74	140	-3 38 C		
	0+3	2.26	1.60	126	-3 16 C		2.27	1.49	144	-3 25 C		
	0+2	2.20	1.55	127	-3 16 C	13.2	2.35	1.55	145	-3 27 C	12.1	
3	0+4	1.46	0.83	71	-5 42 C		1.88	0.88	178	-0 52 C		
	0+3	1.27	1.04	86	-3 39 C	4.8	1.89	1.64	176	+1 54 C	7.7	
4	0+4	1.37	0.68	94	+4 50 C	4.2	0.88	0.03	82	-6 40 C	1.2	
M ₂ First piece							M ₂ Second piece					
0	0+4	3.34	1.26	149	+10 33 A		3.90	1.38	153	+10 14 A		
	0+3	3.65	1.13	154	+10 29 A		3.86	1.46	146	+10 36 A		
	0+2	3.73	1.09	155	+10 27 A		3.80	1.55	148	+10 32 A		
	0+1	3.73	1.07	163	+11 10 A	58.3	3.85	1.57	162	+10 04 A	52.6	
1	0+4	1.50	0.88	46	+7 55 C		2.41	1.52	46	+7 31 C		
	0+3	1.60	1.07	35	+7 14 C		2.54	1.60	32	+7 23 C		
	0+2	1.74	1.53	36	+6 35 C		1.80	1.02	32	+7 01 C		
	0+1	1.72	1.49	38	+6 29 C	16.7	1.72	0.94	34	+6 48 C	14.1	
2	0+4	1.34	1.06	57	+4 06 C		1.76	1.19	51	+3 31 C		
	0+3	1.22	1.09	76	+4 32 C		1.89	1.48	46	+3 26 C		
	0+2	1.19	1.04	82	+4 39 C	11.0	1.81	1.42	46	+3 28 C	18.9	
3	0+4	1.89	1.01	179	-2 24 C		1.43	0.71	71	+2 23 C		
	0+3	1.40	0.78	165	-3 04 C	16.9	1.58	1.14	42	+2 12 C	11.0	
4	0+4	0.71	0.15	14	-6 23 C	2.5	0.90	0.40	142	+1 7 C	3.6	
Perkeris and Accad ⁶												
0		5.1	1.7	153	+9 A							

The dimensions of the ellipses for the baroclinic modes refer to the dimensions at the sea surface.

contours on the neighbouring slope and both ellipses are clockwise. No information can be gained about the vertical structure of the currents on the shelf but other records of tidal currents at the same position as mooring 072 in 1961 and 1962 suggest⁵ that the bottom tidal currents had a magnitude of about 69% of those measured near the surface, that the deep ellipse axes were rotated about 10° clockwise relative to the surface observations and that there was no phase difference between surface and bottom observations. Thus the observations we have made are probably representative of the tidal currents in the entire water column.

The direct comparison with the near surface observations of Cartwright and Woods⁵ is shown in Table 1. The older data were published in the form of mean ellipses for spring and neap tides and we have calculated the separate M_2 and S_2 ellipses from these. No information on the phases of the M_2 and S_2 tidal currents can be extracted from the data of ref. 5.

The results of the decomposition of the currents from mooring 074 into their modal structure are presented in Table 2 for both periodicities and both data pieces. The modal structures remain constant from one piece to the next and because of the lack of energy in the high order modes are insensitive to the number of modes fitted. The dimensions shown in Table 2 refer to the values at the sea surface.

The difference in the energy distributions as a function of mode numbers between the two tidal components is remarkable. For M_2 , between 50 and 60% of the total energy is in the barotropic mode and the remainder is divided almost equally between the first, second and third baroclinic modes. For S_2 less than 10% of the total energy is in the barotropic mode; most of the energy (some 70%) is found in the first baroclinic mode and another 10% or so in the second baroclinic mode (Table 2). This difference in energy distribution is surprising because the modal structures for the two frequencies are almost identical.

Pekeris and Accad⁶ have derived theoretically the barotropic M_2 tidal current ellipses at a variety of grid points. The nearest prediction point to the area in which our observations were made is at about 48° N, 16° W. This is not very close to the observation area but the agreement between the observations and predictions is good. The dimensions of the predicted M_2 ellipse are greater than those observed (Table 2) but Pekeris and Accad found that their predictions of the tidal ranges for the North Atlantic exceeded the observed values (Table 3 of ref. 6); however, the agreement in terms of the shapes, orientations and phases of the observed M_2 tidal ellipses is good.

In spite of the shortness of the current records the results presented here show that the observations of tidal current ellipses for both M_2 and S_2 give consistent results which are in agreement, in the case of the continental shelf observations, with previous observations and, for the deep water, with theoretical predictions of M_2 . The difference in the modal decomposition between the two species is difficult to account for but may result from the local topography and its relation to the directions of propagation of the two tidal components.

The analysis of these data was performed when we were at the Woods Hole Oceanographic Institution. The work was supported by the Office of Naval Research.

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¹ Aanderaa, I. R., *Fixed Buoy Project*, Tech. Rep. No. 16 (NATO Sub-committee on Oceanographic Research, 1964).

² Maagard, L., and McKee, W. D., *Deep-Sea Res.* (in the press).

³ Krauss, W., *Interne Wellen* (Gebrüder Borntraeger, Berlin, 1966).

⁴ Singleton, R. C., *IEEE Trans. on Audio and Electroacoustics*, AU-17 (2), 93 (1969).

⁵ Cartwright, D., and Woods, A. J., *Deutsche Hydrograph. Zeit.*, 16, 64 (1963).

⁶ Pekeris, C. L., and Accad, Y., *Phil. Trans. Roy. Soc., A*, 265, 413 (1969).

Palaeozoic Tethyan Ocean

THE statement by Burrett¹ that there is "no evidence for the existence of a Tethyan Ocean in the Palaeozoic" is incorrect. I refer to a symposium on *Aspects of Tethyan Biogeography* published by the Systematics Association in 1967 (ref. 2). In that volume one of the first articles was by Szalay, entitled *The Tethys in Cambrian Time*. The Bulletin of the American Association of Petroleum Geologists for October 1972 contains an article entitled *Permian Tethys and the Indian Ocean*³. The concept of a Palaeozoic Tethys is so well established in geological literature that a list of references would probably include several thousand titles.

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¹ Burrett, C. F., *Nature*, 239, 155 (1972).

² *Systematics Association, Spec. Publ. 7* (edit. by Adams, C. G., and Ager, D. V.) (Systematics Association, London, 1967).

³ Kamen-Kaye, M., *Bull. Amer. Assoc. Petrol. Geol.*, 50, 1984 (1972).

Reply

THE sentence which Teichert objects to is, when taken in context, a criticism of Nicholas's assumption¹ of the existence of an ocean between Southern Europe and Africa. In geological discussions an ocean may be defined as a deep basin floored by simatic crust generated by the accretion of basaltic material at linear ridges of active volcanism and seismicity ("mid-ocean ridges"). Oceanic growth is accomplished by the process unfortunately known as "seafloor spreading". The Red Sea is an example of an incipient ocean and the Atlantic an example of a fully fledged ocean. This definition would exclude those expanses of salt water usually included in oceans which cover continental crust. These expanses are seas. Clear differentiation between oceans and seas is important in palaeogeographic and tectonic discussions. Nicholas¹ was clearly using his Tethys in the sense defined above with the plate tectonic embellishment of a subducting margin. My communication² arose out of a worldwide, quantitative analysis of Ordovician biogeography (unpublished). The conclusion that the faunal distributions found are best interpreted in terms of oceanic barriers rather than in terms of climate or other ecological factors is also justified elsewhere. Johnson's Provinciality Index³ was used to compare pairs of geographical areas. This index is calculated by dividing the number of common fossil genera by twice the number of endemic genera in the smaller faunal list. Values above one are taken to indicate some degree of co-provincialism. In this study values have ranged from 0–5.2, with most values falling within the range 0.1–0.7. Of all pairs of areas, the comparison between North Africa and Southern Europe gave, by far, the highest values (Fig. 1). On the other hand, indices between Southern Europe and Northern Europe were very low (Fig. 1). Somewhat more sophisticated computerized studies of Cambrian faunal provinces are in preparation by Burrett and Richardson. These

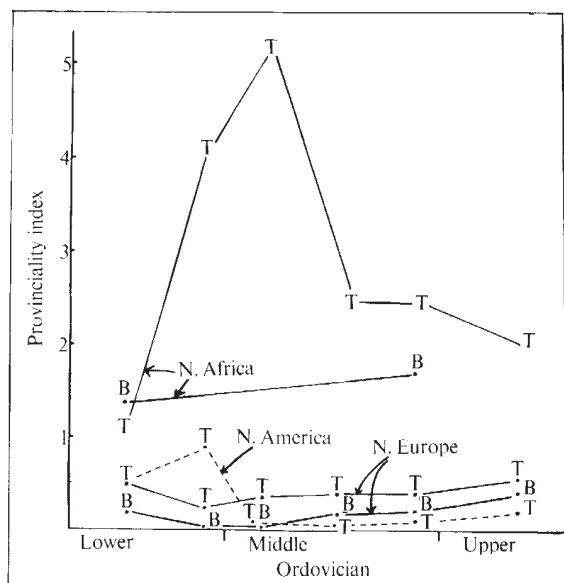


Fig. 1 Plot of provinciality indices against Ordovician time. Southern Europe compared with North Africa, Northern Europe and North America. T, Trilobite; B, brachiopod.

studies further support free faunal interchange between Southern Europe and Africa but show a very clear dichotomy between Northern and Southern Europe. These connexions have been clearly shown by Repina⁴ in a series of maps. In addition the non-existence of archaeocyathids in Northern Europe but their abundance in Southern Europe is further evidence for a Mid-European Ocean but indicates nothing about a Tethyan Ocean. Faunal similarities between Southern Europe and Africa continued through the Palaeozoic⁵ and into the Triassic when the "Tethys itself could have been no more than a minor obstacle to the north-south migration of land animals".⁶ Rifting probably started in the Triassic⁷ and by the Jurassic a reasonably large Tethyan Ocean was in existence⁸.

Thus if an ocean did exist between Southern Europe and Africa it allowed remarkably free passage across it for 600 m.y. Geological evidence for the existence of an ocean on this site is also lacking. Volcanics are sparse and are, for most periods, lacking especially in North Africa. The Upper Cambrian and Ashgillian volcanics that do exist in the Sahara and the volcanics in Spain can be related to subduction along the Proto-Atlantic margin of Southern Europe (Burrett², Fig. 1; and Bard¹⁵). Other evidences such as abundant deep water sediments, though common in the Jurassic Tethyan Ocean, are lacking from the Mediterranean sector of the postulated Palaeozoic Tethys.

That there were epicontinental seas in the Palaeozoic of the Mediterranean region cannot be doubted. These contained very similar faunas in such areas as Turkey, France and Africa. But I have yet to see any evidence for the existence of an ocean separating Africa and Southern Europe. Teichert mentions Sdzuy's paper⁹. Nowhere in that paper, however, does Sdzuy use the term "ocean" but talks of a "seaway". There can be little doubt that to the east an ocean of variable width separated Gondwanaland from the constituent plates of Asia (including South East Asia). The faunal difference between Gondwanaland and Asia and the geological evidence (such as the ophiolites in the Indus Suture zone) are compelling evidence for this ocean. Whether this should be called Tethys is debatable. "Tethyan" has taken on a plethora of meanings. The Tethyan faunal province has for various periods been recognized in the North-west USA, South America, Australia, New Zealand, South East Asia, Yorkshire and Siberia. This is a long way from Suess's original conception of an equatorial Jurassic sea based on an

ancestral Mediterranean^{10,11}. I see no compelling reason or necessity for having a Palaeozoic Ocean separating Southern Europe and Africa. On the other hand, I, like others working from different directions¹²⁻¹⁵, think it necessary to postulate a Mid-European Ocean.

In the 1970s when our concepts of the Earth's behaviour in terms of plate tectonics suggest staggering mobility of continents and the ephemeral nature of oceans it is retrogressive to cling to terms such as Tethys and Tethyan which temporally cover the whole of the Phanerozoic and spatially a large portion of the Earth. At various times they have been used in describing a system of postulated megaseas, a long geosyncline, epicontinental seas, and a variety of faunal provinces caused by a variety of factors. I suggest that the terms Tethys and Tethyan have surpassed their usefulness and should be relegated to the mythological realm from whence they came. Trying to disprove the reality of an ocean named Tethys lying between Southern Europe and Africa is like trying to disprove the existence of leprechauns or God. Like Laplace all I can say is that I have no need for that hypothesis. Those, such as Teichert, who wish to cling to such grossly misused terms should give their reasons as they should for the existence of an ocean, whatever its name, separating Southern Europe and Africa.

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¹ Nicholas, A., *Nature*, **236**, 221 (1972).

² Burrett, C. F., *Nature*, **239**, 155 (1972).

³ Johnson, J. G., *Amer. J. Sci.*, **270** (1971).

⁴ Repina, I., *Akad. Nauk. SSR Siberia Inst. Geol. Geofiz.* (Nauka, Moscow, 1969).

⁵ Boucot, A., Johnson, J. G., and Talent, J. A., *Geol. Soc. Amer. Spec. Pap.*, **119** (1969).

⁶ Charig, A. J., in *Faunal Provinces in Space and Time* (edit. by Middlemiss, F.) (Seel House Press, Liverpool, 1971).

⁷ Trumphy, R., *Bull. Geol. Soc. Amer.*, **71**, 843 (1960).

⁸ Dercourt, J., *Bull. Soc. Geol. France*, **12** (7), 261 (1970).

⁹ Sdzuy, K., in *Systematics Assoc. Publ.* (edit. by Adams, C. G., and Ager, D. V.), **7** (London, 1967).

¹⁰ Sylvester-Bradley, P. C., in *Systematics Assoc. Publ.* (edit. by Adams, C. G., and Ager, D. V.), **7** (London, 1967).

¹¹ Briden, J., and Smith, A., in *The Restless Earth* (edit. by Calder, N.) (BBC, London, 1972).

¹² Johnson, G. A. L., *Internat. Carboniferous Congress* (Krefeld, in the press).

¹³ Tucker, M., thesis, Univ. Reading (1971).

¹⁴ Whittington, H. B., and Hughes, C. P., *Phil. Trans. Roy. Soc.*, **263**, B850, 235 (1972).

¹⁵ Bard, J. P., *Bol. Geol. y Minero*, **82**, 321 (1971).

Photographic Parallax Heights of Infrared Airflow Structures

We recently presented infrared photographs of the night sky which show moving, changing structures¹ and argued that these were due to OH airglow emission. A stringent test of the validity of our assertion is the determination of the height of discrete emission patches using parallax photographs from two widely separated sites. Observations were made during the night of May 2-3, 1973, from Capilla Peak Observatory (elevation 2,855 m) and from a station 20 km west of Albuquerque (1,760 m). The sites are separated by 64.8 km, and the Albuquerque site is located 42.3° west of north from Capilla Peak. Consecutive exposures of 5 min centred about 15° east of north were taken simultaneously at each site with *f*/1.2 cameras from the end of twilight till the beginning of dawn.

Unusual airglow activity occurred from 2330 to 0200 MST in the north-eastern sky. An emission region appeared which

Table 1 Parallax Data May 2-3, 1973

Feature	Z (°)	S (km)	p (°)	h (km)
1 a	80.6	435.8	8.05	88.5
b	79.6	409.7		88.5
2 a	80.2	415.5	8.56	86.6
b	78.8	390.1		89.4
3 a	85.6	719.1	4.68	88.6
b	85.4	689.2		93.6
4 a	84.3	562.6	5.41	88.9
b	83.9	595.1		85.7
Average				(89 ± 3)

a, Determined from Capilla Peak photograph; b, determined from Albuquerque photograph; Z, zenith angle of feature; S, distance of feature from site; p, parallax angle; h, height above mean Earth radius (6,371 km).

was very similar to an auroral drapery. Over the remaining field wide vertical striations developed. The entire pattern was ideal for parallax measurements. From the frame at 0135 MST, four features were measured for both horizontal and vertical parallactic displacement. In Table 1 we list the zenith angle, line of sight distance from the site to the measured feature, parallax angle, and height for each feature. The heights determined are consistent in spite of the large range in distance to the measured features.

Our value of (89 ± 3) km is in good agreement with the rocket measurements of Packer² (90 km) and Evans, Llewellyn and Vallance Jones³ (87 km) for other near infrared OH bands. Other rocket experiments (refs. 4-6 and L. R. Megill, private communication) have yielded values of from 78 to 97 km.

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¹ Peterson, A. W., and Kieffaber, L. M., *Nature*, **242**, 321 (1973).

² Packer, D. M., *Ann. Geophys.*, **17**, 67 (1961).

³ Evans, W. F. J., Llewellyn, E. J., and Vallance Jones, A., *Can. J. Phys.* (in the press).

⁴ Baker, D. J., and Waddoups, R. O., *J. Geophys. Res.*, **72**, 4881 (1967).

⁵ Harrison, A. W., *Can. J. Phys.*, **48**, 2231 (1970).

⁶ Tarasova, T. M., *Space Research III* (North-Holland, 1963).

BIOLOGICAL SCIENCES

Model for Membrane Movements in the Neural Growth Cone

HERE I wish to describe a model for the movements of cellular membranes that is based on observations of the neural growth cone, the structure found at the tips of growing nerve cells¹⁻³. The principal feature of this model is that the contractile proteins responsible for the movement are linked in a polar fashion to membrane surfaces. This gives each region a preferred direction of movement which will be represented by an "arrow" lying in the plane of the membrane. If these arrows are distributed over the inner surface of a cellular structure such as a growth cone, then they can give rise in a simple way to local areas of cellular extension or invagination, and produce many of the features seen by light and electron microscopy.

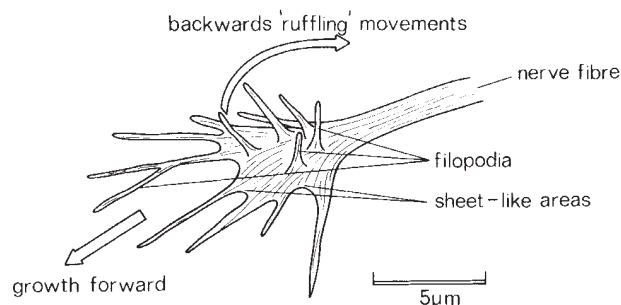


Fig. 1 Sketch of a growth cone (see refs 3 and 4).

The model was suggested by certain features of the ultrastructure of neurones and other cells in culture. The first to be considered is the frequent appearance of cytoplasmic extensions with a constant minimum diameter of between 0.1 and 0.2 μm. On the growth cone, for example, there are numerous filopodia which extend for 10 μm or more⁴ and which for most of their length have a diameter of about 0.15 μm (refs 1, 2) (Fig. 1), while on the surface of embryonic cells in culture⁵ and fibroblasts⁶ there are shorter extensions, called microvilli, of a similar diameter. Experiments to show the protrusion of cytoplasm through filters show that this is blocked at a pore size of 0.15 μm (ref. 7); while even the edges of moving fibroblasts have a thickness of between 0.11 μm and 0.16 μm (ref. 8).

This is interesting because a fixed minimum diameter would imply a common mechanism of formation for these extensions, which at this level would involve basic molecular interactions in the cytoplasm. There is no direct evidence from electron microscopy on how these extensions may grow. They do not contain microtubules, and in fact show little internal structure of any kind. But it is possible, particularly in the case of the growth cone, that they are formed by the incorporation of membranous vesicles at their tips. Abundant elongated vesicles are seen within the broad outspread region of the growth cone, often at the base of filopodia, lying parallel to the long axis^{1,2} (Fig. 2). They are less often seen along the length of a filopodium, where they occupy about a third of the diameter leaving space of about 500 Å on either side.

A distance of 500 Å is particularly significant if related to muscle contraction. Proteins similar to muscle actin and myosin are present in most cells and there is circumstantial evidence that they produce many kinds of cellular movement⁹⁻¹². In skeletal muscle, 500 Å is close to the spacing between adjacent actin or myosin filaments¹³ and is therefore the thickness of a single actin-myosin-actin or myosin-actin-myosin "sandwich". The movement of vesicles within a filopodium could therefore arise quite simply if one of the

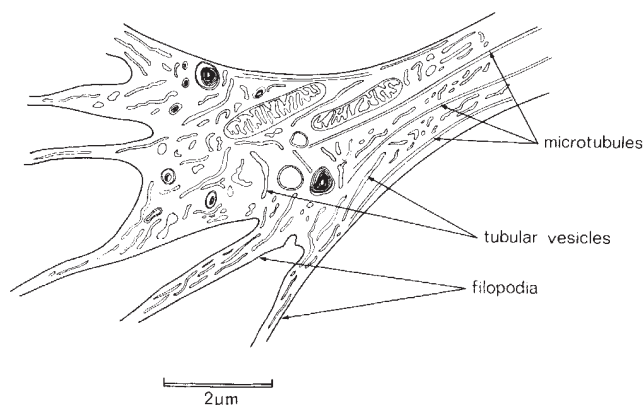


Fig. 2 Sketch of the main ultrastructural features of a growth cone sectioned parallel to the culture surface (see refs 1 and 2).

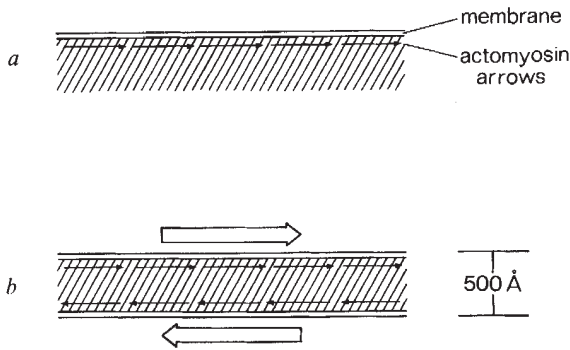


Fig. 3 Membrane polarity. Arrows drawn on the cytoplasmic side of a membrane, as in (a), show the potential it has for movement due to an association with actin or myosin. Actual movement will occur only when two sets of arrows of opposite polarity come within 500 Å, as in (b). Then the membranes will move in the direction indicated by the broad arrows.

two components is present on the opposing surfaces, with the second component in between.

A possible scheme for the extension of filopodia is given in Figs 3 and 4. It is assumed that part of the contractile system, either actin or myosin, lies on the cytoplasmic side of membrane surfaces, and that the other part is freely diffusing in the cytoplasm. The membrane-bound component is distributed in parallel arrays (shown by the arrows) with a tendency for neighbouring molecules to point in the same direction. These will allow local regions to move in the direction of the arrows, but only if another membrane surface of the opposite polarity comes to lie within a 500 Å distance (Fig. 3).

If the arrows in a filopodium, and on the vesicles it contains, are distributed in the sense shown in Fig. 4, then the vesicles will be propelled towards the tip of the filopodium. Once there, we can suppose that they will fuse with the outer membrane so that their outer surface will become part of the inner surface of the filopodium. The membrane arrows, which on the vesicles pointed towards the tip, will reverse their direction because of the eversion and point towards the base. They will therefore orientate so that the transport and fusion of subsequent vesicles will automatically be maintained.

This scheme, therefore, provides a simple way in which long extensions of diameter 0.15 µm could be produced by the incorporation of vesicles which are moved by an actomyosin interaction. It is not given a detailed biochemical interpretation here, although this is possible. The arrows, for example, could be myosin molecules arranged so that their tail-like regions are against the membrane, and which interact with cytoplasmic actin as described in a recent model for smooth muscle contraction¹⁴. Other possible mechanisms will be found in papers on the movements of fibroblasts^{9,15} and the release of synaptic vesicles¹⁶.

The second observation considered is that membrane uptake phenomena, the budding inwards of surface membrane to form small vesicles in the cytoplasm, often seem to occur at the bases of cellular extensions. That this is true in growth cones is suggested by the appearance of coated vesicles, similar to those involved in protein uptake in other cells, close to the bases of filopodia³, and by the evidence that extracellular horse radish peroxidase is taken up by growth cones into clusters of vesicles often near the filopodial base¹⁷. The correlation has been explicitly noted in cultured embryonic cells¹⁸, and electron micrographs of the sheet-like lamellipodia of migrating fibroblasts, for example, show chains of vesicles arising from the proximal side of their base⁸. In kidney proximal tubule, or intestinal epithelia, the uptake of extracellular protein also occurs at the base of the microvilli¹⁹.

I suggest, therefore, that distributed on the surface of a growth cone, or other cellular structure, there are two kinds

of specialized regions; those that produce cellular extensions, filopodia and microvilli, and those that result in membrane uptake or micropinocytosis. These two are linked in some way, so that cellular extension causes, or requires, membrane invagination.

In fact these features arise as a natural consequence of the idea of membrane arrows. The distribution of such polar groups on any surface must give singularities of two kinds (neglecting saddle points), those in which the arrows all point inwards, and its converse. In the scheme described above (Fig. 4), one of these singularities, that of arrows pointing outwards, was present at the tip of a growing filopodium, and was the point at which internal vesicles were thought to fuse. Regions of arrows pointing inwards would, therefore, be produced near the base of filopodia, and it is proposed that membrane invagination occurs at these sites (Fig. 5).

The relationship between the two kinds of site is seen most clearly in a model system in which arrows are distributed on the inside of a spherical membrane surface. If this contains cytoplasm of the correct make-up, the arrows will line up into parallel arrays and automatically produce singularities. One of these will begin to invaginate to form vesicles which will move within the sphere, and against the arrows. The vesicles will accumulate at the other kind of singularity, and here be inserted into the surface and begin to form filopodia (Fig. 6). The properties of the arrows will, therefore, result in the formation of a dynamic system in which filopodial formation and vesicular uptake occur simultaneously at different parts of the sphere.

The last part of the model is added to account for the large-scale movements of growth cones visible in the light microscope, and the continual assembly of new surface membrane as the nerve fibre elongates. Both of these have a directional quality and it is suggested that this is provided by microtubules, which lie along the length of the fibre and extend into the growth cone^{1,2}. These microtubules will be given a polarity of a similar kind to that described for membranes, so that they can interact with vesicles and propel them in a constant distal direction.

The distinctive movements of a growth cone seem to involve a continual stream of surface material from its leading edge back to its base^{3,4}. Filopodia are formed rapidly at the front and then, as they sweep backwards, progressively sink into the cone. These movements strongly resemble those of the "ruffling" membranes seen at the leading edge of migrating fibroblasts, which have been suggested to be a consequence of a cycle of membrane material from its incorporation at the leading edge to its uptake nearer the base^{15,20}. In the growth cone this cycle could be produced by micro-

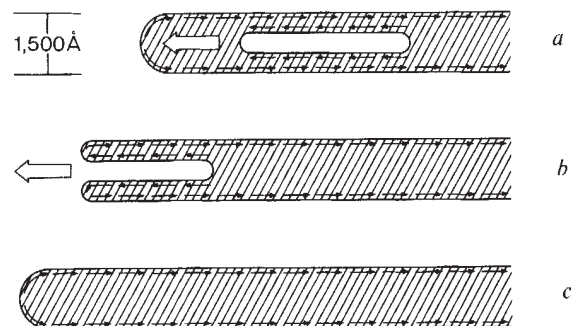


Fig. 4 Growth of a filopodium. A cross-section of a filopodium is shown schematically, together with the actomyosin arrows on its internal surface. In (a), a tubular vesicle (500 Å × 1500 Å) is shown which, because of the orientation of the arrows on its surface and their proximity, is moving in the direction of the broad arrow. In (b), this vesicle has begun to fuse with the distal tip of the filopodium and, in (c), the fusion is complete. Note that the actomyosin arrows automatically assume the correct orientation on the filopodium.

tubules which move internal vesicles to the front, and there favour filopodial formation (while, nearer the base, micropinocytosis would result in the progressive engulfment of filopodia (Fig. 7).

The growth of a nerve fibre must involve the formation of new surface, and there is evidence that this is assembled in the region of the growth cone²¹. Most of the biosynthetic organelles, however, are situated in the cell soma, so that there is likely to be a continual movement of membrane precursors along the neurite. If these precursors are the vesicles seen in electron micrographs along the length of the neurite^{1,2}, then they would in the above way be transported by the microtubules from soma to tip, and there add to the pool of vesicles in the growth cone. Then the assembly of surface could be produced by an adhesion of the cone to the substratum, such that filopodia extended in this region are not retracted by micropinocytosis but, rather, their basal regions are advanced.

The central part of this model, by which it stands or falls, is the polar association of actomyosin proteins with membranes. Actin or myosin, or both, must not only be linked to cellular membranes, but also lie in the kinds of polar organization described above. The first prediction is therefore that such arrangements will eventually be seen by electron microscopy. Another prediction concerns the mode of growth of many cellular extensions, and in particular the filopodia of growth cones. Given adequate fixation and sampling procedures, examples should be seen in which these contain chains of vesicles along their length and evidence of fusion at their tips (as in Fig. 4). If this is not found, or if evidence for alternative modes of extension are

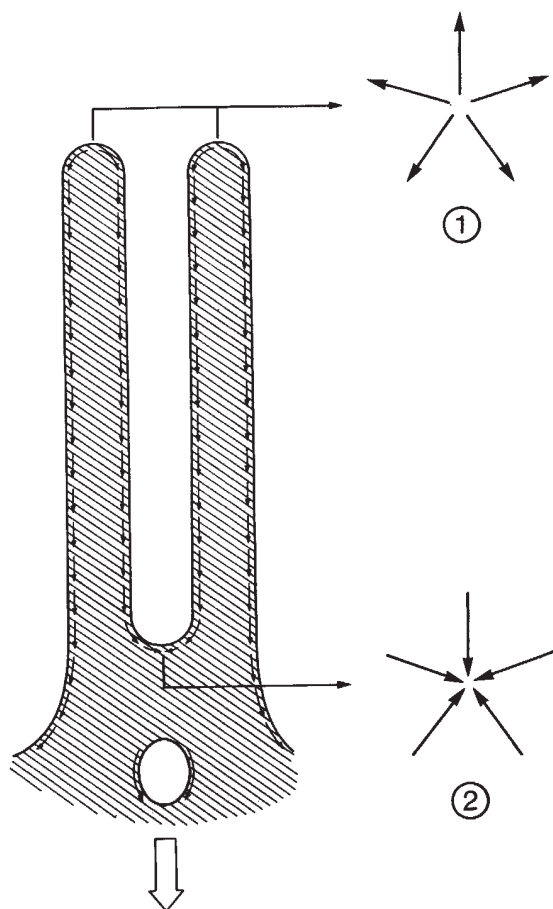


Fig. 5 Sites of vesicle formation. Two adjacent filopodia are shown, their actomyosin arrows distributed as in Fig. 4. At the tips the arrows form a discontinuity in which they all point outwards (1). At their base a complementary pattern will be formed in which all arrows point inwards (2). It is assumed that at such a region, invagination to form vesicles occurs.

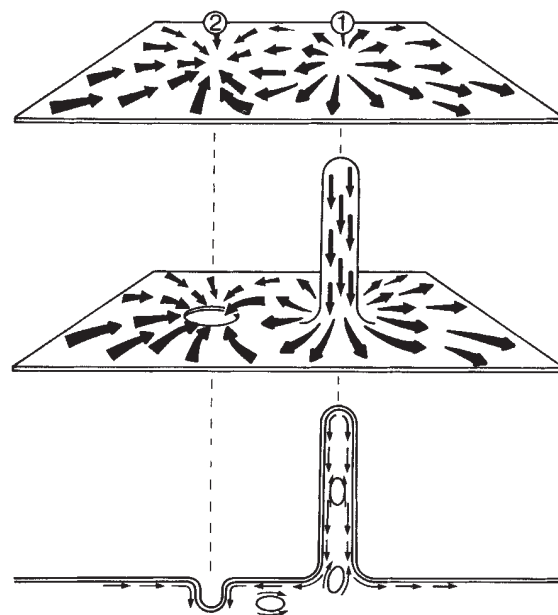


Fig. 6 Behaviour of model system. *a*, A region of membrane is shown together with the actomyosin arrows on its cytoplasmic surface. These are distributed so as to give two kinds of singularity, (1) and (2) (see Fig. 5). *b* and *c*, The membrane at (2) has begun to bud inwards to form vesicles, which move against the surface arrows to (1). Here they are incorporated into filopodia as shown in Fig. 4.

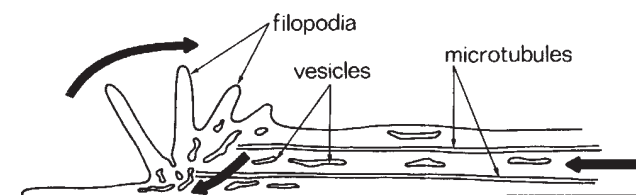


Fig. 7 Schematic cross-section of a neurite tip. Membrane vesicles move in a constant, distal, direction by interaction with microtubules. In the nerve fibre this interaction will propel vesicles from their site of synthesis in the cell body to the tip. In the growth cone this motion will favour the assembly into filopodia at the leading edge, and membrane ingress into vesicles nearer its base. A cycle of surface structures of the kind observed will in this way be set up.

obtained, then the basic idea of membrane polarity, but little else, could remain.

I have tried to present this model concisely because it is so speculative and cannot be correct in every detail. But in outline it is the kind of explanation that must be found: one which shows how a precise molecular mechanism can be coordinated in different parts of a cell to give large-scale movements, and which takes as evidence regular features of an otherwise disordered and complex cytology.

So many colleagues have helped me in the preparation of this manuscript that I cannot name them here, but I must acknowledge my debt to their generous and perceptive criticism.

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Received February 20, 1973.

- ¹ Bunge, M. B., *J. Cell Biol.*, **56**, 713 (1973).
- ² Yamada, K. M., Spooner, B. S., and Wessells, N. K., *J. Cell Biol.*, **49**, 614 (1971).
- ³ Bray, D., *J. Cell Biol.*, **56**, 702 (1973).
- ⁴ Nakai, J., *Amer. J. Anat.*, **99**, 81 (1956).
- ⁵ Fisher, H. W., and Cooper, T. W., *J. Cell Biol.*, **34**, 569 (1967).

- ⁶ Follett, E. A. C., and Goldman, R. D., *Exp. Cell Res.*, **59**, 124 (1970).
- ⁷ Wartiovaara, J., Lehtonen, E., Nordling, S., and Saxén, L., *Nature*, **238**, 407 (1972).
- ⁸ Abercrombie, M., Heaysman, J. E., and Pegrum, S. M., *Exp. Cell Res.*, **67**, 359 (1971).
- ⁹ Huxley, H., *Nature*, **243**, 445 (1973).
- ¹⁰ Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Ludeña, M. A., Taylor, E. L., Wrenn, J. T., and Yamada, K. M., *Science*, **171**, 135 (1971).
- ¹¹ Jahn, T. L., and Bovee, E. C., *Physiol. Rev.*, **49**, 793 (1969).
- ¹² Bettex-Galland, M., and Lüscher, E. F., *Adv. Protein Chem.*, **20**, 1 (Academic, NY and London, 1965).
- ¹³ Huxley, H., *Biochim. Biophys. Acta*, **12**, 387 (1953).
- ¹⁴ Small, J. V., and Squire, F. M., *J. Mol. Biol.*, **67**, 117 (1972).
- ¹⁵ Harris, A., *Locomotion of Tissue Cells*, CIBA Symposium (in the press).
- ¹⁶ Berl, S., Puszkun, S., and Nicklas, W. J., *Science*, **179**, 441 (1973).
- ¹⁷ Bunge, M., *Anat. Rec.*, **175**, 280 (1973).
- ¹⁸ Cornell, R., *Exp. Cell Res.*, **57**, 86 (1969).
- ¹⁹ Graham, R. C., and Karnovsky, M. J., *J. Histochem. Cytochem.*, **14**, 291 (1966).
- ²⁰ Abercrombie, M., Heaysman, J. E. M., and Pegrum, S. M., *Exp. Cell Res.*, **62**, 389 (1970).
- ²¹ Bray, D., *Proc. US Nat. Acad. Sci.*, **65**, 905 (1970).

Rapid Immunological Method for Diagnosis of Natural Scrapie in Sheep

SCRAPIE may be diagnosed rapidly in the experimental mouse by inoculating some tissue (brain, spleen, muscle, for example) into a guinea-pig and measuring the degree to which the guinea-pig lymphocytes become sensitized to scrapie mouse brain (or spleen) as compared with normal mouse brain (or spleen)¹. Lymphocyte sensitization is measured by the macrophage electrophoresis migration (MEM) test²⁻⁴. This in principle depends on the observation that when lymphocytes are brought into contact with specific antigen they elaborate some material (involving protein synthesis⁵) which causes normal guinea-pig peritoneal macrophages to travel more slowly in an electric field. Thus normal guinea-pig macrophages are used as an indicator system for lymphocyte-antigen interaction. The percentage slowings induced by such interactions are calculated and a high percentage indicates lymphocyte sensitization to the test antigen.

As the guinea-pig injected experimentally with scrapie

Table 1 Macrophage Electrophoresis Migration*

Normal Scrapie OND† Clinical details				Normal Scrapie OND† Clinical details			
Newcastle (Longbenton)—Veterinary Investigation Centre (January 11, 1973)				8 Normal brain	14.1		Born April 15, 1971; 22 months; natural clinical scrapie, January 1972
1 Normal brain	2.8		3-yr-old normal	Scrapie brain	23.2		
Scrapie brain	3.4			Normal spleen	4.5		
Normal spleen	2.4			Scrapie spleen	13.0		
Scrapie spleen	3.2			S-ND (brain)	9.1		
S-ND‡ (brain)	0.6			S-ND (spleen)	8.5		
S-ND (spleen)	0.8			Moredun February 7, 1973			
2 Normal brain	15.6		3-yr-old natural scrapie	9 Normal brain	2.6		Old ram; born 1966; injected scrapie 1967.
Scrapie brain	23.3			Scrapie brain	5.3		Nil
Normal spleen	4.5			Normal spleen	2.1		
Scrapie spleen	12.4			Scrapie spleen	4.7		
S-ND (brain)	7.7			S-ND (brain)	2.7		
S-ND (spleen)	7.9			S-ND (spleen)	2.6		
Moredun Institute, Edinburgh (January 30, 1973)				10 Normal brain	3.4		Senile ram; born 1964; injected scrapie 1964.
3 Normal brain	2.9		Born April 16, 1970; 34 months not affected by scrapie	Scrapie brain	4.9		Nil
Scrapie brain	3.6			Normal spleen	2.9		
Normal spleen	2.9			Scrapie spleen	4.8		
Scrapie spleen	3.7			EF	4.6? ageing		
S-ND (brain)	0.7			S-ND (brain)	1.5		
S-ND (spleen)	0.8			S-ND (spleen)	1.9		
4 Normal brain	2.6		Born April 7, 1970; 34 months not affected by scrapie	11 Normal brain	14.9		Born 1969; injected scrapie November 10, 1971; now clinical scrapie
Scrapie brain	3.3			Scrapie brain	22.9		
Normal spleen	2.6			Normal spleen	3.1		
Scrapie spleen	3.3			Scrapie spleen	12.2		
EF	1.5			EF	18.7		
S-ND (brain)	0.9			S-ND (brain)	8.0		
S-ND (spleen)	0.7			S-ND (spleen)	9.1		
5 Normal brain	2.7		Born April 3, 1971; 22 months not affected by scrapie	12 Normal brain		16.3	Natural border disease; born 1970
Scrapie brain	3.4			Scrapie brain		17.7	
Normal spleen	2.5			Normal spleen		2.8	
Scrapie spleen	3.3			Scrapie spleen		4.7	
S-ND (brain)	0.7			EF		16.3	
S-ND (spleen)	0.8			S-ND (brain)		1.4	
6 Normal brain		14.2	Born April 22, 1971; 22 months; natural clinical scrapie October 1972	S-ND (spleen)		1.9	
Scrapie brain		23.4		13 Normal brain		16.2	Experimental border disease; born 1970
Normal spleen		4.2		Scrapie brain		17.4	
Scrapie spleen		12.6		Normal spleen		2.6	
EF		17.6		Scrapie spleen		4.4	
S-ND (brain)		9.2		S-ND (brain)		1.2	
S-ND (spleen)		8.4		S-ND (spleen)		1.8	
7 Normal brain		15.3	Born April 10, 1971, 22 months; natural clinical scrapie; December 1972	14 Normal brain		9.5	Cerebrocortical necrosis recovered by January 20, 1973; born 1968
Scrapie brain		23.1		Scrapie brain		12.3	
Normal spleen		4.5		Normal spleen		2.0	
Scrapie spleen		12.8		Scrapie spleen		5.3	
S-ND (brain)		7.8		EF		11.0	
S-ND (spleen)		8.3		S-ND (brain)		2.7	
				S-ND (spleen)		3.3	

* Figures are percentage macrophage slowing in MEM test^{2,4}, calculated as $(t_e - t_c/t_c) \times 100$ where t_e = migration time of macrophages when antigen present; t_c = migration time of macrophages when no antigen present (control).

† OND, other neurological diseases.

‡ S-ND, scrapie-normal difference.

material shows increased reactivity of its lymphocytes to scrapie as opposed to normal tissues, the same might be true of a sheep affected with natural scrapie. Experiments were set up with lymphocytes prepared from the blood of normal sheep, scrapie sheep with both the natural and experimental forms and sheep with such other neurological diseases (OND) as were available for study. These comprised two cases of border disease and one of cerebro-cortical necrosis. In addition some old sheep injected with scrapie material but which had not shown any clinical illness were also studied. Results are given in Table 1. In normal animals the difference in lymphocyte response to scrapie compared with normal tissue (scrapie-normal difference: S-ND) does not exceed 2.7%. When either brain or splenic tissue is used as antigen, S-ND is much higher in both natural and experimental scrapie (animals numbers 1, 6, 7, 8, 11). In those with border disease, either natural or experimental, and in the animal with recovered cerebro-cortical necrosis it was within the normal range. These preliminary results suggest that determination of the S-ND in response to mouse scrapie and normal brain or splenic tissue may be used to make a diagnosis of scrapie in the sheep.

A study of human neurological disease including multiple sclerosis, neurosyphilis, kuru, Jakob-Creutzfeldt disease and miscellaneous disease not especially associated with gliosis, indicated that a high S-ND may be associated with astroglial overgrowth in the brain⁶. The same high S-ND may be induced in guinea-pig lymphocytes, however, by the injection of scrapie non-nervous tissue (for example, muscle) in which there are, of course, no astroglial cells. It may well be, therefore, that astrogliosis is only the cerebral morphological manifestation of some change induced in cell membranes (associated with altered antigenicity) by the scrapie agent⁶⁻⁸.

Border disease in sheep is not especially characterized by astrogliosis. The degree of astrocyte hypertrophy in scrapie may depend upon the "strain" of scrapie agent used and perhaps also on the breed of sheep employed. The MEM test is highly discriminatory and might be of value in distinguishing different "strains" of scrapie agent.

In a few cases sensitization to encephalitogenic factor (EF)⁹ has been tested. As expected, normal sheep showed very little, but animals with scrapie, border disease or cerebro-cortical necrosis showed the sensitization which would be expected as a consequence of brain destruction¹⁰.

Clearly a more extensive study of sheep with a variety of neurological diseases needs to be carried out, especially any which is characterized by astroglial proliferation, as these too may be expected to show a high S-ND⁶. Such study may indeed establish the test described as a rapid and simple means of excluding scrapie without the need to kill the animal and prolonged bioassay; as it might prove of considerable economic importance, further independent study on a larger scale seems desirable.

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¹ Field, E. J., and Shenton, B. K., *Nature*, **240**, 104 (1972).

² Field, E. J., and Caspary, E. A., *Lancet*, ii, 1337 (1970).

- ³ Field, E. J., and Caspary, E. A., *J. clin. Path.*, **24**, 179 (1971).
⁴ Caspary, E. A., and Field, E. J., *Br. med. J.*, **2**, 613 (1971).
⁵ Caspary, E. A., *Nature new Biology*, **231**, 24 (1971).
⁶ Field, E. J., and Shenton, B. K., *Brain* (in the press).
⁷ Gibbons, R. A., and Hunter, G. D., *Nature*, **215**, 1041 (1967).
⁸ Hunter, G. D., *J. infect. Dis.*, **125**, 427 (1972).
⁹ Caspary, E. A., and Field, E. J., *Ann. NY Acad. Sci.*, **122**, 182 (1965).
¹⁰ Caspary, E. A., and Field, E. J., *Eur. Neurol.*, **4**, 257 (1970).

Embryonic and Adult Chymotrypsinogens of Chicken Pancreas

BEEF and pig pancreas contain chymotrypsinogens A, B and C, homologous proteins whose activation products have different substrate specificities¹⁻⁴. Chymotrypsinogens of the A and B type have been found in several vertebrate species⁵ whereas the C type chymotrypsinogen seemed to be restricted to the pig and cow²⁻⁴. An investigation in this laboratory⁶ showed that chick pancreas secretory granules contain three chymotrypsinogens, called chymotrypsinogens 1, 2 and 3. The specificities of the activation products of chymotrypsinogens 1 and 2 resembled those of bovine chymotrypsinogens B and A respectively^{2,6,7}. Because of the small relative amounts of chymotrypsinogen 3 in the extracts, the specificity of its activation product could not be determined with certainty, but some similarities to mammalian chymotrypsin C were detected⁶. As the data of a later investigation⁸ suggested that embryonic chick pancreas might contain relatively large amounts of chymotrypsinogen 3, the chymotrypsinogens in the developing chick pancreas were further studied. This report shows that the specificity of chick embryo chymotrypsin 3 is similar to that of mammalian chymotrypsin C. We also show that chymotrypsinogen 3 is the major chymotrypsinogen species during embryonic development but becomes a minor species after hatching, when chymotrypsinogens 1 and 2 become prominent.

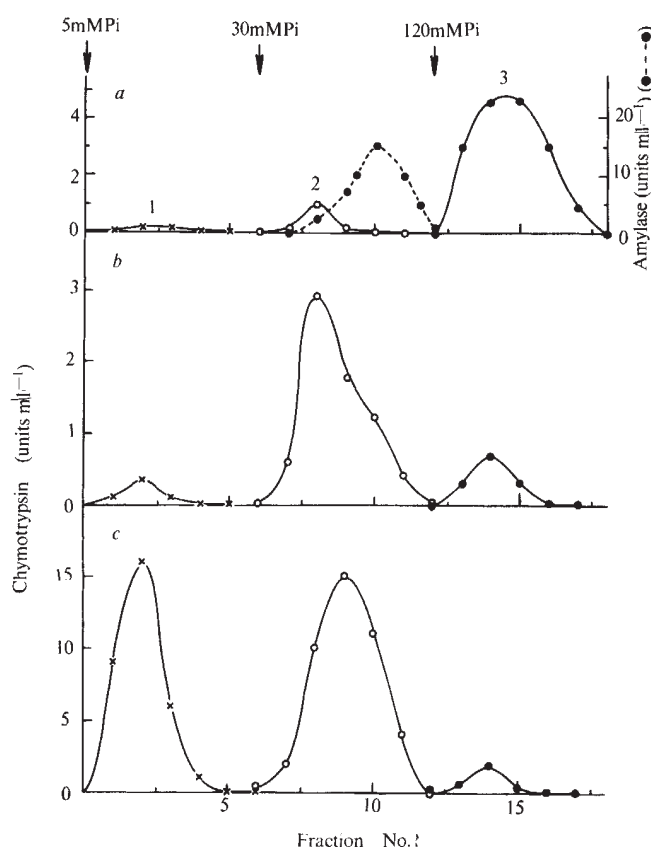
Table 1 Specificity of Chymotrypsin 3 from Embryonic Chick Pancreas

Substrate	Methanol concentration (% v/v)	Relative activity
ATEE	0	100
BLEE	0	156
BTEE	2.5	255
ATryEE	30	3

Relative activity=(rate of hydrolysis of substrate/rate of hydrolysis of ATEE) × 100. Chymotrypsinogen 3 was separated from other chymotrypsinogens of 19-d-old embryo pancreas as described in the legend to Fig. 1. Activities of the peak fraction of chymotrypsinogen 3 were measured after activation with trypsin. For other details see Fig. 1 and ref. 6.

Chromatography on DEAE cellulose of the pancreatic secretory proteins of chick embryos and newly hatched chicks shows that, as in the case of older chickens⁶, there are three species of chymotrypsinogen present (Fig. 1). The activities of the activation products of the three chymotrypsinogens were tested on N-acetyl-L-tyrosine ethyl ester (ATEE), on N-acetyl-L-tryptophan ethyl ester (ATryEE) in 20% methanol and on N-benzoyl-L-leucine ethyl ester (BLEE). The specificities of the activation products of chymotrypsinogens in the first two peaks corresponded respectively with the specificities of chymotrypsin 1 (type B) and chymotrypsin 2 (type A) described previously⁶. Table 1 shows that the specificity of the third chymotrypsin(ogen) peak resembles that of mammalian chymotrypsin(ogen) C, which has a high relative activity on BLEE and a low relative activity on ATryEE^{2,6}. The activities of chymotrypsin 3 on BLEE and N-benzoyl-L-tyrosine ethyl

Fig. 1 Chromatography of chymotrypsinogens from embryonic and newly-hatched chick pancreas. Elution patterns of chymotrypsinogens from, *a*, 10-d-old embryo; *b*, 1-d-old chick (developmental age 22 d) and, *c*, 4-d-old chick (developmental age 25 d). The arrows show the points at which the phosphate buffer concentrations were changed to the concentrations indicated. Embryos and chicks were White Leghorn $\times$ New Hampshire. Exportable enzymes were extracted from the pancreases by incubation *in vitro* with a secretory stimulant²¹. The pancreases were incubated in 50 ml stoppered Erlenmeyer flasks with 5.0 ml of medium which consisted of Hanks' balanced salt solution containing glucose and vitamins as in Dulbecco's modified Eagle's medium, 20 μ g ml⁻¹ of crystalline soybean trypsin inhibitor (Worthington Biochemical Corporation) and 10⁻⁵ M carbamyl choline. Each flask contained either thirty pancreases from 19-d-old embryos or four pancreases from 1- or 4-d-old chicks. The flasks were gassed with 95% O₂ and 5% CO₂ and incubated at 80 r.p.m.⁻¹ in a rotating shaker bath at 37° C. After incubation for 2 h, when 30–50% of the exportable enzymes in the pancreas had been secreted, the medium containing the secreted enzymes and zymogens was collected and dialysed overnight against 5 mM phosphate buffer pH 8.0. The dialysed extract was chromatographed on microgranular DEAE cellulose (Whatman DE 52) previously equilibrated with 5 mM phosphate buffer pH 8.0. Extracts of the pancreas of 19-d-old embryos which contained about 0.4 mg of protein were chromatographed on 0.3 $\times$ 1.5 cm columns at a flow rate of 5–10 ml h⁻¹ and 1.0 ml fractions were collected. Extracts of pancreas from 1- and 4-d-old chicks which contained about 2.5 and 4 mg of protein respectively were chromatographed on 0.7 $\times$ 5.0 cm columns at a flow rate of 30–50 ml h⁻¹ and 5.0 ml fractions were collected. The fractions were diluted five times with 0.05 M Tris-HCl buffer pH 8.0 and were activated by incubating with 20 μ g ml⁻¹ of trypsin for 90 min at 25° C. In order to eliminate any errors which might arise from the overlapping of peaks, chymotrypsin(ogen)s 2 and 3 were estimated with substrates which were highly specific for each (that is ATryEE in 20% methanol (○) for chymotrypsinogen 2 and BLEE (●) for chymotrypsinogen 3). As chymotrypsinogen 1 has no specific substrate and was widely separated from the other peaks its activity was determined with ATEE (×). Enzyme activities on the various substrates were measured as described previously⁶ with a Gilford 2000 spectrophotometer.



ester (BTEE) are 1.5 times and 2.5 times higher, respectively, than its activity on ATEE. On the other hand, the relative activity of this chymotrypsin on ATryEE in 20% methanol is only 3% of its activity on ATEE.

Although, according to its specificity, the third chymotrypsinogen peak described here resembles the chymotrypsinogen 3 of chickens found previously⁶, it appears to have a different elution pattern as it is eluted after amylase rather than before it. In an earlier study on 6-week-old chickens the chymotrypsinogen 3 peak coincided with a peak of procarboxypeptidase A but no such procarboxypeptidase A peak

was observed in our investigation. The difference in the chromatographic behaviour of chymotrypsinogen 3 in the two investigations may thus be attributable to the existence of chymotrypsinogen 3 in the form of a complex with procarboxypeptidase A in pancreatic extracts of 6-week-old chickens but in free form in extracts of embryonic pancreas. Bovine chymotrypsinogen C occurs in the form of a complex with procarboxypeptidase A⁴.

Figure 1 shows that the relative activities of the three types of chymotrypsin(ogen) change during development. Up to the nineteenth day chymotrypsinogen 3 is the major species

Table 2 Developmental Changes in Chick Pancreas Chymotrypsin(ogen)s

Developmental age (d)	Total units per pancreas Chymotrypsin(ogen)			Specific activity (units mg ⁻¹ protein) Chymotrypsin(ogen)			Ratio of activities Chymotrypsin(ogen)		
	1	2	3	1	2	3	1	2	3
16	<0.001	0.012	0.19	<0.001	0.017	0.27	<0.0005	0.06	1
19	0.10	0.32	4.6	0.07	0.23	3.3	0.02	0.07	1
22	2.4	33	9.1	0.16	2.4	0.64	0.27	3.6	1
25	86	122	12.0	4.8	7.3	0.74	7.2	10.2	1
31	132	107	16.0	6.5	5.3	0.76	8.3	6.7	1

The data summarize the results of DEAE cellulose chromatographic separations of chick pancreas zymogens similar to those shown in Fig. 1. The data for each developmental age are the mean of two to three experiments. Enzymes and zymogens were extracted from the pancreases by stimulation *in vitro* as described under Fig. 1. Only a portion of each enzyme was extracted by this procedure. Because of the lack of specific methods of assay and because of the presence of soybean trypsin inhibitor in the extracts, it was impossible to determine directly the percentage of each chymotrypsinogen secreted. The percentage of amylase secreted was therefore used to estimate the percentage of each chymotrypsinogen secreted. A previous study⁸ showed that the percentage of amylase secreted by chick pancreas *in vitro* was the same as the percentage of other enzymes and zymogens secreted during the same period of incubation. The amount of each chymotrypsin originally present in the pancreases from which extracts were prepared was calculated as follows. Total amount of chymotrypsinogen in pancreases = [amount of chymotrypsinogen eluted from column] $\times$ [(amylase secreted + amylase remaining in pancreases)/(amylase secreted)]. The calculation was based on the assumption that here, as in previous experiments⁶, the chymotrypsinogens were quantitatively eluted from the columns. Activity per pancreas was the total activity of each enzyme originally present in the secretion system divided by the number of pancreases. Protein was determined by the method of Lowry *et al.*¹⁹ and amylase activity according to Bernfeld²⁰. Specific activity is the total amount of enzyme originally present in the pancreas/total protein originally present in the pancreas.

found. After 22 d (1 d after hatching) chymotrypsinogen 2 has become the major species, while at 25 d (4 d after hatching) chymotrypsinogens 1 and 2 are equally prominent (Fig. 1). Table 2 shows in more detail the changing ratios of chymotrypsinogens 1, 2 and 3. The ratio of the species of chymotrypsinogen seems to change because the accumulation of each species slows down at a different stage of development. Thus, the accumulation of chymotrypsinogen 3 falls off after 19 d of development while the accumulation of chymotrypsinogen 2 slows down after 22 d and that of chymotrypsinogen 1 slows down only after 25 d. The specific activity of chymotrypsinogen 3 reaches a peak at 19 d of development. This emphasizes its dominance in the embryonic pancreas and the decline in its relative amount after hatching. At 19 d chymotrypsinogen 3 constitutes about 3% of the total protein. These results illustrate how the relative amount of specific proteins in the cells is affected by the different times during development at which their accumulation ceases.

These data are reminiscent of changes in the patterns of isoenzymes during development, of which various examples are known⁹⁻¹². There are also marked similarities between the changes in the ratios of the different species of chymotrypsinogen during development and developmental changes in the ratios of haemoglobin species¹³⁻¹⁵. In both cases molecular species which are dominant in the embryo become minor components later during development, and it is likely that chicken chymotrypsinogens 1, 2 and 3 are homologous molecules^{14,15}. In the chicken, the changes in the haemoglobin species during development seem to be correlated with changes in the type of cell synthesizing haemoglobin^{13,16}. In the mammal, on the other hand, single red cells contain both foetal and adult haemoglobin^{14,15}. This raises the question of whether the change in the pattern of chymotrypsinogens in the chick pancreas is attributable to changes in the pattern of gene expression in the same cell or to the emergence of a new dominant cell type with a different pattern of gene expression. At present there are no data to shed light on this problem.

The physiological significance of the shift in the ratio of chymotrypsinogen species during development is obscure. As significant amounts of amylase (about 5% of the total amount in the pancreas) have been found in the intestine of 17-d-old embryos (Cohen, unpublished experiments) there seems to be some pancreatic secretion at this stage of development. The role of this secretion may be to digest albumin entering the intestine from the stomach and/or yolk reaching the intestine from the yolk sac¹⁷. As egg proteins contain an unusually high percentage of leucine¹⁸, the predominance in pancreatic juice of chymotrypsin(ogen) C, which has a high relative activity on peptide bonds adjacent to leucyl residues, may be advantageous.

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- ¹ Hartley, B. S., and Shotton, D. M., *The Enzymes*, third ed. (edit. by Boyer, P. D.), 3, 323 (Academic, New York, 1971).
- ² Folk, J. E., and Schirmer, E. W., *J. Biol. Chem.*, **240**, 181 (1965).
- ³ Gratecos, D., Guy, O., Rovey, M., and Desnuelle, P., *Biochim. Biophys. Acta*, **175**, 82 (1969).
- ⁴ Brown, J. R., Greenshields, R. N., Yamasaki, M., and Neurath, H., *Biochemistry*, **2**, 867 (1963).
- ⁵ Wilcox, P. E., in *Methods in Enzymology* (edit. by Perlmann, G. E., and Lorand, L.), **19**, 64 (Academic, New York, 1970).
- ⁶ Zelikson, R., Eilam-Rubin, G., and Kulka, R. G., *J. Biol. Chem.*, **246**, 6115 (1971).
- ⁷ Prahl, J. W., and Neurath, H., *Biochemistry*, **5**, 2131 (1966).
- ⁸ Cohen, A., Heller, H., and Kulka, R. G., *Develop. Biol.*, **29**, 293 (1972).
- ⁹ Markert, C. L., and Ursprung, A., *Developmental Genetics* (Prentice-Hall, Englewood Cliffs, N.J., 1971).

- ¹⁰ Doane, W. W., in *Problems of Development: RNA in Development* (edit. by Hanly, E. W.), 73 (University of Utah Press, Salt Lake City, 1969).
- ¹¹ Eppenberger, H. M., Eppenberger, M., Richterich, R., and Aebi, H., *Develop. Biol.*, **10**, 1 (1964).
- ¹² Leberherz, H. G., *Develop. Biol.*, **27**, 143 (1972).
- ¹³ Wilt, F. H., in *Advances in Morphogenesis*, **6**, 89 (1967).
- ¹⁴ Baglioni, C., in *Molecular Genetics* (edit. by Taylor, J. H.), Part 1, 405 (Academic, New York, 1963).
- ¹⁵ Ingram, V. M., *The Hemoglobins in Genetics and Evolution* (Columbia University Press, New York, 1963).
- ¹⁶ Hagopian, H. K., Lippke, J. A., and Ingram, V. M., *J. Cell Biol.*, **54**, 98 (1972).
- ¹⁷ Romanoff, A. L., *The Avian Embryo* (Macmillan, New York, 1960).
- ¹⁸ Romanoff, A. L., and Romanoff, A. J., *The Avian Egg* (Wiley, New York, 1949).
- ¹⁹ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
- ²⁰ Bernfeld, P., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **1**, 149 (Academic, New York, 1955).
- ²¹ Kulka, R. G., Heller, H., and Marchaim, U., in *Secretory Mechanisms of Salivary Glands* (edit. by Schneyer, L. H., and Schneyer, C. A.), 254 (Academic, New York, 1967).

Modification of Tumour Growth with a Defined Glycoprotein Antigen

MANY pathogens escape the body's immune system because their surface antigenic determinants elicit an immune response that is too weak, temporary, or of the wrong type. A plausible solution to this type of problem is to put the antigenic determinant specific for the pathogen on a suitable carrier and present it to the host immune system in a way that will elicit a protective immune response of the right type, time course, and strength. This has been called the "chemical vaccine" approach¹.

Cancer is an example of this type of problem. The cell surface receptor sites that bind tumour specific lectins such as wheat germ agglutinin² and concanavalin A^{3,4} are potential tumour-specific antigenic determinants. Wheat germ agglutinin has maximum specificity for di-N-acetyl- β -chitobiosyl-asparagine⁵, and an antigen designed to elicit an immune response specific for this structure demonstrated several properties expected of a synthetic tumour-specific antigen⁶ including conferring a degree of protection against transplanted and chemically induced tumours in animals immunized against it⁵. This structure is found in only one place in mammalian systems, at the carbohydrate protein linkage of the asparagine-linked class of glycoproteins⁷. If the tumour-specific wheat germ agglutinin receptor site is a glycoprotein as this suggests, it is a rather unusual member of the asparagine-linked class in that it has a very much shorter sugar chain than is typical⁷. As the short sugar chain may have arisen on the surfaces of tumour cells by a general mechanism that might also generate short sugar chain glycoproteins of other classes, an antigen containing a short sugar chain belonging to the serine and/or threonine linked class of glycoproteins was prepared and tested as a synthetic tumour specific transplantation antigen.

The freezing point depressing glycoproteins from the blood serum of the Antarctic fish *Trematomus borchgrevinki* contain an appropriate disaccharide unit, β -galactopyranosyl-(1,4)- α -N-acetylgalactosaminylthreonine⁸, and only a few modifications were required to convert a sample of these glycoproteins (obtained from A. L. DeVries and Y. Lin, purified by chromatography on DEAE-cellulose⁹) to a form suitable for testing as a tumour-specific antigen (Fig. 1). As these glycoproteins are not immunogenic in rabbits (A. L. DeVries, unpublished results), they were converted to a form that could be aggregated with methylated bovine serum albumin (MBSA) to yield a complex that would be expected¹⁰ to be more immunogenic. The glycoproteins were treated with enough sodium periodate to oxidize about 20% of the galactose residues to give dialde-

hyde units that were further oxidized with bromine water⁸ to dicarboxylic acid units. The resulting glycoprotein derivatives are negatively charged and can be aggregated with methylated bovine serum albumin (MBSA). As this glycoprotein contains a sugar unit for every three amino acids⁹, it may exist in solution as a polypeptide coil completely covered with sugar units. To ensure that some of the antigenic determinants involved in the immune response elicited by the antigen could consist of the complete disaccharide unit with adjacent amino acids, and not exclusively the terminal sugars of adjacent sugar chains, about two-thirds of the sugar units were removed under β -elimination conditions followed by hydrogenation of the resulting double bond⁹. The resulting antigen, called antigen FA, was used as a complex with an equal weight of MBSA, designated FA(MBSA).

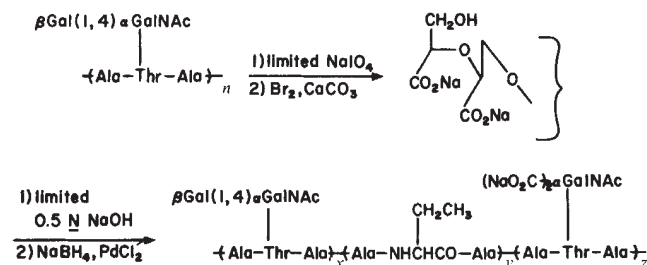


Fig. 1 Preparation of antigen FA. $n=15, 24$, or 30 ; $x \approx 4n/15$; $y \approx 2n/3$; $z \approx n/15$.

The effectiveness of the antigen complex was evaluated in a series of experiments using two groups of ten Fisher F344 rats (Microbiological Associates) immunized with aqueous solutions of FA(MBSA) or of the control antigen MBSA at the same concentration emulsified in three volumes of complete Freund's adjuvant. Each animal was given an intradermal injection of 0.2 ml of emulsion in each groin, boosted in the same manner 2 weeks later. Three weeks after the booster injection they were challenged with a subcutaneous injection in the flank of a sufficient number of Fisher rat dimethylbenzanthracene-induced mammary adenocarcinoma cells (DMBA No. 8)¹¹, to give a progressively growing tumour (generally 1×10^5 cells) that was measured at intervals after challenge. The cells were maintained in culture in Dulbecco's modification of Eagle's medium supplemented with 10% foetal bovine serum (Flow Laboratories) and 10% tryptose phosphate broth (Difco). Experiments were carried out using immunization with doses of 1 μg , 5 μg , 10 μg , 50 μg , and 100 μg of FA as the complex FA(MBSA).

Protection against the transplanted tumour was observed with the lowest level of immunizing antigen tested (Fig. 2A, 1 μg , at 13 d after challenge, Student's $t=1.82$, $P<0.05$; at 21 d, $t=0.99$, $P<0.20$) and enhancement of tumour growth was observed with higher levels of immunizing antigen (Fig. 2B, 5 μg , at 14 d after challenge, Student's $t=2.50$, $P<0.025$; at 21 d, $t=2.09$, $P<0.050$; Fig. 2C, 10 μg , at 13 d, Student's $t=2.50$, $P<0.025$; at 20 d, $t=2.07$, $P<0.050$). These results are consistent with antigen FA functioning as a synthetic analogue of the tumour-specific transplantation antigens which have been shown in animal systems to confer protection against transplanted tumours in some immunizing conditions and to enhance tumour growth in other immunization conditions⁶. Lower levels of a glycoprotein antigen favour the production of a cell mediated immune response¹², which should be protective⁶ if FA is functioning as a synthetic tumour-specific

transplantation antigen, whereas higher levels of a glycoprotein antigen¹² favour the production of a greater humoral component to the total immune response. If FA is functioning as a tumour-specific transplantation antigen, a humoral immune

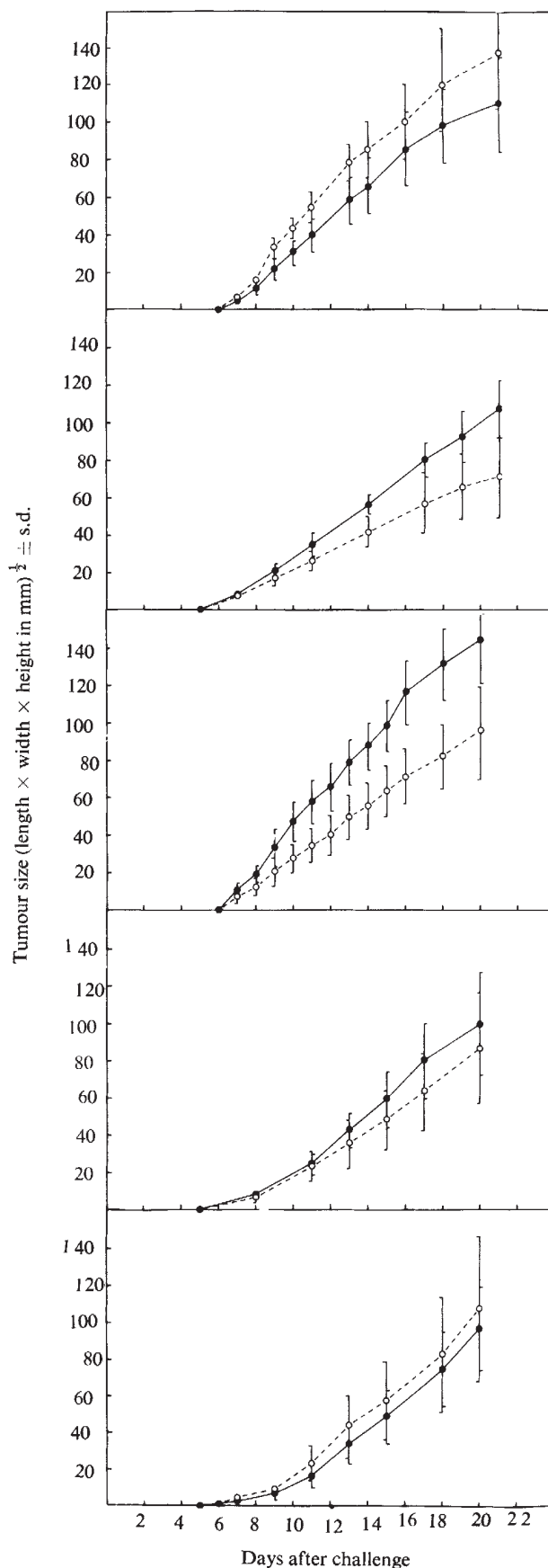


Fig. 2 Effect of immunization of Fisher rats against the indicated levels of antigen FA and challenge with 5×10^5 DMBA No. 8 tumour cells. The average of square roots of the products of the dimensions of the growing tumours was plotted rather than the average of the geometric means because these values give more nearly linear growth curves in this system. A, 1 μg FA; B, 5 μg FA; C, 10 μg FA; D, 50 μg FA; E, 100 μg FA. $\circ$, MBSA; $\bullet$, FA(MBSA).

response might be expected to cause enhancement of tumour growth⁶. It has not yet been established whether the lack of significant protection or enhancement of tumour growth at the two highest levels of immunizing antigen (Fig. 2D, 50 µg, at 13 d after challenge, Student's $t=0.59$, $P<0.30$; Fig. 2E, 100 µg, at 13 d after challenge, Student's $t=0.71$, $P<0.25$) is attributable to the development of immunological paralysis to the immunizing antigen¹³ or to the production of a second type of humoral immune response analogous to the "deblocking" activity described by Bansal and Sjogren¹⁴. These degrees of protection and enhancement are comparable with those obtained in this system with antigen A⁵, the antigen designed on the basis of wheat germ agglutinin specificity (W. T. Shier, unpublished results).

The effect of antigen FA has also been evaluated in Balb/c mice for protection against an intraperitoneal injection of 2.5×10^6 XS63.5 Balb/c myeloma cells (the number of cells that gives approximately 50% mortality in control mice) as described previously⁵. Immunization with 1 µg of antigen FA as the MBSA complex by the same immunization schedule as was used in rats, except that one-half the volume of emulsion was used, conferred a marginally significant ($\chi^2=2.03$, $P<0.15$) degree of protection in terms of a decreased incidence of mortality caused by tumours (Table 1) in the animals immunized with FA.

Table 1 Effect of Immunization of Balb/c Mice with 1 µg of Antigen FA Complexed with MBSA

No. of XS63.5 cells 2.5×10^6	Incidence of tumours	
	FA(MBSA)	MBSA
	8/25 (32%)	14/25 (56%)

An alternative explanation for the observed degrees of protection and enhancement of tumour growth is that the immune response to FA exerts its effect on the whole immune system of the immunized animal, causing general immunological stimulation with low levels of FA and general immune suppression with high levels of FA. This does not appear to be the case.

Balb/c mice were immunized with a range of doses of FA(MBSA) or MBSA by the immunization schedule described above then challenged with a second antigen, sheep red blood cells, and the immune response assessed 4 d later using the Mishell-Dutton modification of the Jerne plaque assay¹⁵ (Table 2). As the differences observed are within the precision range of the assay, it is doubtful that immunization against FA exerts any significant effect on the immune response to cell surface antigens. The lack of general immunological suppression also suggests that there is no significant attack by the immune response to FA on normal dividing cells, as considerable division of normal lymphocytes is involved in the process of mounting an immune response to an antigen.

Table 2 Effect of Antigen FA on the Immune Response to a Second Antigen, Sheep Red Blood Cells

Primary antigen	Antigen dose (µg)	Average No. of plaque forming cells per 10^5 spleen cells $\pm$ s.e.
FA(MBSA)	100	91.6 ± 2.6
MBSA	100	101.2 ± 2.3
FA(MBSA)	10	96.6 ± 4.7
MBSA	10	91.1 ± 2.2
FA(MBSA)	1	99.5 ± 2.5
MBSA	1	95.1 ± 2.9

Clearly, the degrees of enhancement and protection observed with antigen FA are not striking, but the observation that the growth of tumours in animals immunized with different amounts of FA is modified in directions consistent with FA functioning as a tumour specific transplantation antigen lends

some credence to the suggestion that the disaccharide structure β Gal(1,4) α GalNAcThr, as well as the wheat germ agglutinin binding disaccharide structure di-N-acetylchitobiosylasparagine⁵, may be present in different numbers or different distributions on the surfaces of tumour cells than on the surfaces of normal cells.

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- ¹ Foster, A. O., *Sci. News*, **98**, 280 (1970).
- ² Burger, M. M., and Goldberg, A. R., *Proc. US Nat. Acad. Sci.*, **51**, 359 (1967).
- ³ Inbar, M., and Sachs, L., *Nature*, **223**, 710 (1969).
- ⁴ Inbar, M., and Sachs, L., *Proc. US Nat. Acad. Sci.*, **63**, 1418 (1969).
- ⁵ Shier, W. T., *Proc. US Nat. Acad. Sci.*, **68**, 2078 (1971).
- ⁶ Hellstrom, K. E., and Hellstrom, I., *Ann. Rev. Microbiol.*, **24**, 373 (1970).
- ⁷ Marshall, R. D., and Neuberger, A., in *Advances in Carbohydrate Chemistry and Biochemistry*, **25** (edit. by Tipson, R. S.), 407 (Academic Press, New York, 1970).
- ⁸ Shier, W. T., Lin, Y., and DeVries, A. L., *Biochim. Biophys. Acta*, **263**, 406 (1972).
- ⁹ Komatsu, S. K., DeVries, A. L., and Feeney, R. E., *J. Biol. Chem.*, **245**, 2901 (1970).
- ¹⁰ Plescia, O. J., Braun, W., and Palczuk, N. C., *Proc. US Nat. Acad. Sci.*, **52**, 279 (1964).
- ¹¹ Dennert, G., and Lennox, E. S., in *Microenvironmental Aspects of Immunity, Advances in Experimental Medicine and Biology*, **29** (edit. by Janković, B. D., and Isaković, K.), 157 (Plenum Press, New York, 1973).
- ¹² Salvin, S. B., *J. Exp. Med.*, **107**, 109 (1958).
- ¹³ Felton, L. D., Kauffmann, G., Prescott, B., and Ottinger, B., *J. Immunol.*, **74**, 17 (1955).
- ¹⁴ Bansal, S. C., and Sjogren, H. O., *Int. J. Cancer*, **9**, 490 (1972).
- ¹⁵ Mishell, R. I., and Dutton, R. W., *J. Exp. Med.*, **126**, 423 (1967).

Methadone Induced Mortality as a Function of the Circadian Cycle

We have previously reported¹ the results of our study demonstrating a circadian variation in the susceptibility of groups of rats to a lethal dose of methadone. The statistical significance of this finding has been criticized by Argyle², who proposed the binomial test as a more appropriate indicator of the significance of the data. Additional experiments not only support the conclusions of the earlier work, but have also provided an opportunity for a more rigorous statistical test of our hypotheses.

In our initial study, six groups of rats were randomly selected and maintained on a standardized regimen which included a 12 h light-dark cycle. Rats were then given a previously determined dose of methadone at 4 h intervals throughout the circadian cycle, and mortality statistics were collected. The results indicated a circadian variation in the lethality of methadone, with the rats most sensitive at the onset of their activity period, 2100 h, and most resistant toward the latter portion of their activity period, at 0500 h. These data were then treated statistically in a similar manner to previous studies³ dealing with the circadian variability of other pharmacological agents.

The choice of the χ^2 test as a good estimation of the statistical significance of a multinomial distribution is well documented⁴ and is not "incorrect". A much more specific method of statistically analysing data relating to circadian variation has

been clearly set forth by Halberg⁵ and is referred to as the cosinor technique but, because of the lack of sufficient data points at this early part of our work, this was not applicable. Argyle's choice of a binomial approach to the data is an exact but tedious statistical test in this case, which ignores the cyclical expectation of the data format. However, assuming the naivety of this expectation, Argyle's calculations of the probabilities involved are correct and are indicative of the marginal statistical significance found by the χ^2 at only two points on the curve. Realizing the preliminary nature of our report, we have been carrying out follow-up studies, one of which has direct bearing on this question.

Table 1 Susceptibility of Rats to Methadone Hydrochloride Administered on Two Consecutive Days

Time of day	n	Survivals	Mortalities	Mortalities (%)	Standard error (%)
0500	20	12	8	40	± 11
2100	20	4	16	80	± 9

Based on our previous data of a peak mortality at 2100 h, and a trough at 0500 h, these two points were selected for further study to establish a more precise test of the statistical significance of the circadian variability. According to the protocol outlined in our study, forty rats were randomly selected and maintained at the same environmental specifications. Methadone hydrochloride was administered on two consecutive days at 2100 h and 0500 h, and the data shown in Table 1 were collected. The dose of methadone was reduced from our original dosage level of 47 mg kg⁻¹ to 40 mg kg⁻¹ in order to gain greater sensitivity in evaluating this phenomenon. The χ^2 test in this case is statistically significant at $P < 0.025$. A more precise statistical analysis appropriate to this experimental design is the Fisher exact probability test⁴ which delivered a two-tailed significance level of 0.022. It would therefore appear that the hypothesis of circadian variability of methadone lethality can be accepted at least for these two points on the curve. It remains to be seen how general this specific function will be throughout different species, and how cyclical the data will be according to Halberg's technique as new data are gathered.

It is now possible to combine the data from the two experiments in the interest of increasing the statistical significance as Argyle has suggested but there is reluctance to do this because of the possible interference of other biological factors including seasonal variation.

Our decision to report the initial portion of our studies was based not only on the statistical significance at that time, but also on its more immediate significance to the scientific community as a stimulus to further interest and research concerning methadone. With the addition of these new data, even greater justification can be provided for undertaking further explorations of the circadian variables affecting the physiological response to methadone.

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¹ Lenox, R. H., and Frazier, T. W., *Nature*, **239**, 397 (1972).

² Argyle, E., *Nature*, **242**, 332 (1973).

³ Scheving, L. E., Vedral, D. F., and Pauly, J. E., *Nature*, **219**, 621 (1968).

⁴ Armitage, P., *Statistical Methods in Medical Research* (Blackwell Scientific Publications, Oxford and Edinburgh, 1971).

⁵ Reinberg, A., and Halberg, F., *Ann. Rev. Pharmacol.*, **11**, 455 (1971).

Continued Expression of H-Y Antigen on Male Lymphoid Cells resident in Female (Chimaeric) Mice

REJECTION of male skin grafts by female mice of the same inbred strain is due to male-specific (H-Y) antigen^{1,2} (review³). As the only functions so far definitely traced to the Y chromosome are concerned with sex determination, a question arises as to whether the expression of H-Y antigen on male cells is secondary to development of the male phenotype, or whether a gene (or genes) on the Y chromosome specifies H-Y antigen (either directly or under regulation of an autosomal locus) independently of other sex-related characteristics. The fact that H-Y antigen is found on all male tissues that have been

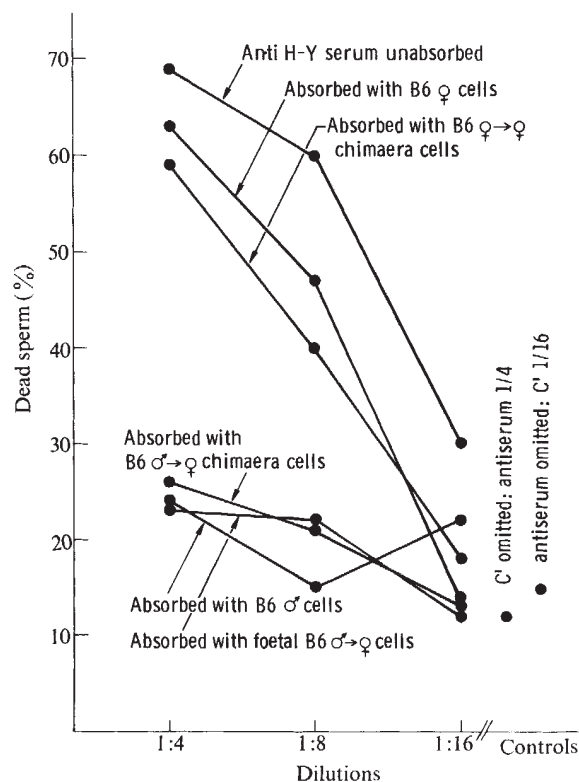


Fig. 1 A representative test showing absorption of H-Y antibody by lymphoid cells from a C57Bl/6 (B6) male-to-female radiation chimaera. Anti-H-Y serum was obtained from B6 females which had rejected two successive male skin grafts, and then received five weekly intraperitoneal inoculations of 150×10^6 B6 male spleen and lymph node cells. Aliquots of anti-H-Y serum (diluted 1:4) were absorbed with lymph node and spleen cells from each chimaera, for 30 min in an ice bath. The absorbed sera were then titrated for residual cytotoxic H-Y antibody, using horse serum diluted 1:16 as a complement (C') source and B6 epididymal sperm as a target (method of Goldberg *et al.*⁴). The sperm were stained with trypan blue. The results shown above are titrations after absorption with cells from single donors. The slight fall in titre produced by absorption with female cells, and by absorption with cells from female-to-female chimaeras, is attributable to non-specific factors involved in the absorption procedure.

tested, rather than being restricted to sex-related tissues^{3,4}, is perhaps an indication that it is not directly concerned with the attributes of male sexuality. Its appearance on male but not on female cells might instead be a fortuitous consequence of the lack of crossing over between the X and Y chromosomes; in this case, any change in cell surface antigenicity brought about by mutation of genes on the Y chromosome would automatically be transmitted only to males.

Many experiments involving hormonal manipulation and transplantation *in vivo*⁵⁻⁸ have been designed to settle the question whether H-Y antigen would be expressed on male cells transferred to female recipients, but with no decisive result. Now that an adequate serological test has been devised for detecting H-Y antigen⁴, a definitive answer is possible. As we report here, this method shows unequivocally that H-Y antigen continues to be expressed on male haemopoietic cells after their transfer to female recipients, indicating that expression of H-Y antigen in these cells is autonomous and does not depend upon their continued residence in the male environment.

Anti-H-Y sera are prepared by immunizing female mice with cells from males of the same inbred strain. These sera, in the presence of complement, are cytotoxic for sperm⁴ and male epidermal cells⁹. H-Y antigen also occurs on cells from the spleen and lymph nodes (for example) but in a quantity too small for detection by the cytotoxicity test⁴; therefore serological demonstration of H-Y antigen on such cells has depended on their capacity to absorb the cytotoxic activity of anti-H-Y antibody specifically against sperm and epidermal cells⁴.

In our experiments, male-to-female chimaeras were produced by lethally irradiating virgin female (90 to 120 days of age) C57Bl/6 (B6) mice (850 r whole body X-irradiation) and restoring them with intravenous injections of approximately 100×10^6 spleen and bone marrow cells from adult B6 males, or with injections of 25×10^6 liver cells from 17-d-old foetal B6 males. Ten to twelve weeks later, the chimaeras were killed, and cells from their lymph nodes and spleens were tested for the presence of H-Y antigen by serological absorption.

In every case, the absorption tests have shown the presence of H-Y antigen. Figure 1 illustrates one of four such tests, including controls for specificity. While it is thus clear that the male environment is not essential to the maintenance of H-Y antigen on haemopoietic cells, it is still possible that differentiation pathways peculiar to the male are essential to initiation of H-Y antigen expression, which is then irreversible. Further serological tests directed to that point are in progress.

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- ¹ Eichwald, E. J., and Silmsker, C. R., *Transplant Bull.*, **2**, 148 (1955).
- ² Hauschka, T. S., *Transplant Bull.*, **2**, 154 (1955).
- ³ Gasser, D. L., and Silvers, W. K., *Adv. Immunol.*, **15**, 215 (1972).
- ⁴ Goldberg, E. H., Boyse, E. A., Bennett, D., Scheid, M., and Carswell, E. A., *Nature*, **232**, 478 (1971).
- ⁵ Engelstein, J. M., *Proc. Soc. Exp. Biol. Med.*, **126**, 907 (1967).
- ⁶ Poláková, M., and Vojtěšková, M., *Folia Biol. (Praha)*, **14**, 93 (1968).
- ⁷ Silvers, W. K., *J. Exp. Med.*, **128**, 69 (1968).
- ⁸ Silvers, W. K., Billingham, R. E., and Sanford, B. H., *Nature*, **220**, 401 (1968).
- ⁹ Scheid, M., Boyse, E. A., Carswell, E. A., and Old, L. J., *J. Exp. Med.*, **135**, 938 (1972).

Ovarian Control of Vitellogenin Synthesis by the Fat Body in *Aedes aegypti*

INSECT yolk proteins, or vitellogenins, are synthesized and secreted by the fat body¹⁻⁴, and are sequestered from the haemolymph by the developing oocytes⁵⁻⁷. Hormonal involvement in this process has been suggested by our work with the fat body of *Aedes aegypti*^{4,8}, and we now report that a factor, produced by the ovary, acts directly on the fat body to activate and maintain synthesis.

Ligature experiments have shown that a hormone from the head controls egg development in *A. aegypti* and is necessary for 4-8 h after a blood meal^{9,10}. This "egg development neurosecretory hormone"^{11,12} originates in the median neurosecretory cells of the brain^{11,12}. Egg development was determined by measuring the size of the oocyte after various treatments, but because egg development is a complex process it is not clear what aspect was being controlled by the hormone. We have developed a method for assaying vitellogenin synthesis by the fat body, enabling us to distinguish the role of the hormone in regulating vitellogenin synthesis from other possible functions it may have in egg development.

Table 1 Effect of Neck Ligature on Vitellogenin Synthesis by the Fat Body

Time of neck ligature (h after blood meal)	c.p.m. in antibody precipitate/ µg tissue protein
0	5.0
0.5	5.4
1	15.8
2	10.4
3	19.1
4	40.7
5	55.2
6	71.3
8	87.8
10	117.6
Unligatured fed control	119.2
Unligatured unfed control	4.7

At various times after a blood meal, female mosquitoes were ligated at the neck and decapitated. 18 h after feeding, the fat body from ten females was dissected free of other tissues (but left attached to the abdominal wall) and incubated in 0.1 ml of an organ culture medium⁸ containing 5 µCi of ³H-phenylalanine. After 3 h of incubation the medium was withdrawn and the tissues were washed twice with 0.05 ml of medium. The fat body was removed and frozen. The medium was combined with the washings (total volume, 0.2 ml) and centrifuged at 12,500g for 10 min. To 0.05 ml aliquots of the supernatant were added 0.01 ml of carrier yolk protein (1 mg ml⁻¹) and 0.05 ml of antiserum to purified yolk protein. The antigen-antibody precipitate was allowed to form at 37° C for 1 h and at 0° C for 1 h (ref. 27) and was then deposited on 'Millipore' filters, washed and counted in a liquid scintillation counter⁸. The fat body tissues were homogenized and analysed for protein by the method of Lowry²⁰. The data are averages from two separate experiments.

Females were ligatured at various times after feeding and vitellogenin synthesis by the fat body was measured 18 h after the blood meal in the organ culture system described in Table 1. Ligature at the neck within 30 min of feeding prevented synthesis. When the ligature was applied at 1-3 h there was a low level of synthesis, and with ligature from 4 h on, synthesis increased rapidly to control values (Table 1). These data agree well with the results of Gillett¹⁰ and demonstrate that a brain hormone is involved in controlling vitellogenin synthesis.

The brain hormone could be acting directly on the fat body, or through some other organ such as the ovary itself. To investigate the latter possibility we ovariectomized females, gave them a blood meal, and then assayed synthesis by the fat body as before. Ovariectomy prevented vitellogenin synthesis (Table 2). This effect is humoral, and not nervous, as shown by the partial restoration of synthesis when donor ovaries

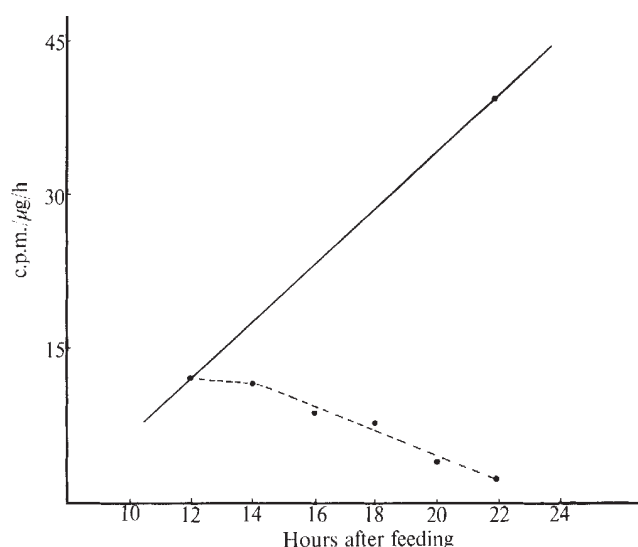


Fig. 1 Effect of ovariectomy 12 h after the blood meal on vitellogenin synthesis. Female mosquitoes were fed and ovariectomized 12 h later. Vitellogenin synthesized by the fat body was assayed at 2 h intervals thereafter (see Table 1). The data are means of two replicates of ten fat bodies each. —, Control females, not ovariectomized; ---, ovariectomized females.

were implanted into an ovariectomized female fed 1 h previously (Table 2). It seems, therefore, that an ovarian hormone is involved in controlling vitellogenin synthesis, and we decided to investigate this further.

Table 2 Effect of Ovariectomy on Vitellogenin Synthesis by the Fat Body

	n	c.p.m. in antibody precipitate/ µg tissue protein
Sham operated fed control	5	185.2 ± 11.5
Sham operated unfed control	4	5.0 ± 1.1
Ovariectomized fed	4	5.8 ± 0.5
Ovariectomized fed + 3 h ovary	6	56.2 ± 7.4

Five-day-old females were ovariectomized through slits between the fifth and sixth abdominal segments and allowed to recover for 24 h. They were then given a blood meal. Eighteen hours later vitellogenin synthesis by the fat body was assayed as described in Table 1. Implanted ovaries were from donors fed 3 h previously. The ovaries were injected into the abdomen of ovariectomized females, fed 1 h previously, through a slit between the fourth and fifth abdominal segments by means of equipment described by Ursprung²¹. The data are shown with their standard errors.

We first looked at the kinetics of the effect of ovariectomy. Females were ovariectomized 12 h after the blood meal and vitellogenin synthesis was assayed at 2 h intervals thereafter. The effect of ovariectomy was apparent within 2 h; the normal increase in the rate of synthesis ceased and was followed by a steady decline to the level found in an unfed animal (Fig. 1). These data indicate that the ovary must be continuously present for the fat body to synthesize vitellogenin at an increasing rate. They further suggest that the ovary acts directly on the fat body.

To test the latter possibility we incubated fat body in the presence and absence of ovaries (Fig. 2). When fat body was incubated alone the rate of synthesis was constant for about 3 h and then declined. When ovaries were present in the culture medium, however, the rate of synthesis increased with time, as it does *in vivo* after the blood meal (ref. 7 and Fig. 1). This suggests that with the ovaries present in culture, conditions *in vitro* are analogous to those *in vivo*.

In this experiment it is unlikely that the fat body increased its rate of synthesis because the ovary was acting as a sink, removing vitellogenin (or some other factor) from the medium. The medium was replaced every hour in both control and experimental preparations. This in itself created a sink, periodically removing all synthesized materials and creating the same situation for both groups. Any result, therefore, could not be due to differential ovarian uptake. Furthermore, to demonstrate the effect of the ovary on vitellogenin synthesis it was necessary to incubate the medium with ovaries for 1 h before adding it to the fat body preparation. This requirement indicates that the factor is being secreted by the ovary.

Table 3 Effect of the Ovarian Factor on the Body from Unfed Females

	n	c.p.m. h ⁻¹
Unfed fat body + unfed ovary	4	142 ± 9
Unfed fat body + 18 h ovary	4	1,484 ± 506
18 h ovary	4	36 ± 6

Fat body from unfed females was incubated in the presence of ovaries taken from donors fed 18 h earlier, or ovaries from unfed donors. The incubation was continued for 12 h. Ovaries from 18 h donors were also incubated alone. Vitellogenin synthesis was assayed as described in Table 1. The data are shown with their standard errors.

These data show that the ovarian factor maintains the rate of vitellogenin synthesis *in vivo* and *in vitro*. Does the ovary also activate synthesis? Fat body from unfed females which does not synthesize vitellogenin^{4,7} can be used to answer this question. Table 3 shows that when fat body from unfed females is incubated with 18 h ovaries vitellogenin synthesis is indeed turned on. As controls, we incubated 18 h ovaries alone (to eliminate the possibility of contamination by active fat body) and fat body from unfed females with their own (unfed) ovaries.

These observations can be summarized as follows; the blood meal has been shown to trigger the release of the egg

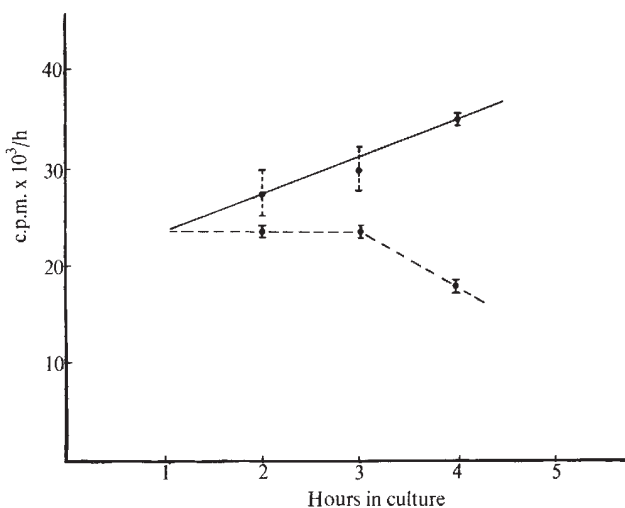


Fig. 2 Effect of the ovary on synthesis of vitellogenin *in vitro*. The fat body was taken from forty females 18 h after the blood meal and incubated in groups of ten in 0.1 ml of medium containing ³H-phenylalanine. Two groups were kept continuously in the presence of ovaries in medium that was also preincubated with ovaries for 1 h before use. The ovaries were taken from the same (18 h) females. The two control groups were incubated without ovaries. The medium was changed every hour. The first hour's sample was discarded. Vitellogenin synthesis was assayed as described in Table 1. The data are the means from three experiments ± standard error. —, Fat bodies incubated with ovaries; ---, fat bodies incubated without ovaries.

development hormone from the brain¹⁰⁻¹². After 4-8 h vitellogenin synthesis by the fat body proceeds independently of the brain. The events during this period are obscure; it is clear, however, that the ovary begins to produce a humoral factor which activates and maintains the normal rate of vitellogenin synthesis by the fat body.

The presence, in mosquitoes, of a humoral factor from the ovary controlling vitellogenin synthesis is unusual among insects. In another dipteran, the fleshfly *Sarcophaga*, ovariectomy leads to an accumulation of vitellogenin in the haemolymph¹³, as it does in several other insects^{14,15}. Ovarian control of the fat body has been proposed for *Drosophila*¹⁶, but supporting evidence has been difficult to obtain¹⁷. This apparently unique system among the insects is perhaps understandable when one considers that most mosquitoes and other blood sucking insects (such as *Rhodnius*) are unusual in the obligatory relationship between the blood meal and oogenesis. Each blood meal normally leads to the development of a batch of eggs. In mosquitoes the entire process takes only 3 d and can begin again after another blood meal. Such a system would need a control not required in other insects where feeding and egg production are continuous. This is emphasized by the evidence^{18,19} for another ovarian hormone in mosquitoes which emanates from the finished eggs, inhibiting development of the penultimate oocytes.

Although rare in insects, ovarian hormones which activate vitellogenin synthesis are well known in vertebrates. Thus in the chicken²² and in the toad *Xenopus*^{23,24}, and more recently in fish²⁵, *in vitro* synthesis of vitellogenin by liver slices taken from animals injected with oestrogen has been demonstrated. We note that it has not been possible to initiate vitellogenin synthesis *in vitro* by oestrogen. Thus our data showing activation of synthesis *in vitro* are exceptional. An intriguing result of our continuing work on the ovarian factor is the demonstration that injected ecdysone stimulates vitellogenin synthesis²⁶.

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- ¹ Pan, M. L., Bell, W. J., and Telfer, W. H., *Science*, **165**, 393 (1969).
- ² Engelmann, F., *Science*, **165**, 407 (1969).
- ³ Brookes, V. J., *Develop. Biol.*, **20**, 459 (1969).
- ⁴ Hagedorn, H. H., and Judson, C. L., *J. Exp. Zool.*, **182**, 367 (1972).
- ⁵ Telfer, W. H., *J. Biophys. Biochem. Cytol.*, **9**, 747 (1961).
- ⁶ Roth, T. F., and Porter, K. R., *J. Cell Biol.*, **20**, 313 (1964).
- ⁷ Stay, B., *J. Cell Biol.*, **26**, 49 (1965).
- ⁸ Hagedorn, H. H., Fallon, A. M., and Laufer, H., *Develop. Biol.*, **31**, 285 (1973).
- ⁹ Gillett, J. D., *Ann. Trop. Med. Parasitol.*, **50**, 375 (1956).
- ¹⁰ Gillett, J. D., *Nature*, **180**, 656 (1957).
- ¹¹ Lea, A. O., *J. Insect Physiol.*, **13**, 419 (1967).
- ¹² Lea, A. O., *Gen. Comp. Endocrinol., Suppl.*, **3**, 602 (1972).
- ¹³ Wilkins, J. L., *J. Insect Physiol.*, **15**, 1015 (1969).
- ¹⁴ Telfer, W. H., *J. Gen. Physiol.*, **37**, 539 (1954).
- ¹⁵ Thomas, K. K., and Nation, J. L., *Biol. Bull.*, **130**, 254 (1966).
- ¹⁶ Doane, W. W., *J. Exp. Zool.*, **146**, 275 (1961).
- ¹⁷ Doane, W. W., in *Developmental Systems: Insects* (edit. by Counce, S. J., and Waddington, C. H.), **2**, 291 (Academic Press, London, 1973).
- ¹⁸ Lea, A. O., *J. Med. Entomol.*, **9**, 99 (1972).
- ¹⁹ Else, J., and Judson, C. L., *J. Med. Entomol.*, **9**, 527 (1972).
- ²⁰ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

- ²¹ Ursprung, H., in *Methods in Developmental Biology* (edit. by Wilt, W. H., and Wessells, N. K.) (Crowell, New York, 1967).
- ²² Heald, P. J., and McLachlan, R. M., *Biochem. J.*, **94**, 32 (1965).
- ²³ Rudack, D., and Wallace, R. A., *Biochim. Biophys. Acta*, **155**, 299 (1968).
- ²⁴ Wallace, R. A., and Jared, D. W., *Develop. Biol.*, **19**, 498 (1969).
- ²⁵ Plack, P. A., and Fraser, N. W., *Biochem. J.*, **121**, 857 (1971).
- ²⁶ Fallon, A. M., and Hagedorn, H. H., *Amer. Zool.*, **12**, 322A (1972).
- ²⁷ Horwitz, M. S., and Scharff, M. D., in *Fundamental Techniques in Virology* (edit. by Habel, K., and Salzman, N. P.), 297 (Academic Press, New York, 1969).

Sterilization of Rodent and other Pests using a Synthetic Oestrogen

THE development of rat and mouse strains resistant to warfarin and other anticoagulants has emphasized the need for other means of rodent control. The most important drawback of both anticoagulants and acute poisons is that failure to achieve complete eradication can result in accelerated breeding by the surviving animals and the population being rapidly restored to its original size.

The use of chemosterilants has therefore been proposed (refs. 1-3 and Marsh, R. E., unpublished data) in the expectation that an inability to replace the numbers lost through natural mortality will lead to the extinction of an infestation. The use of synthetic oestrogens for this purpose has received particular attention, although mestranol, the 3-methyl ether of 17 α -ethinyl oestradiol, has proved rather disappointing under field conditions⁴, as the rats developed an aversion to the oestrogen-containing bait. In a more recent field trial with quinoestrol⁵, the corresponding 3-cyclopentyl ether, repeated applications produced a significant reduction in the incidence of pregnancy despite a slow decline in bait acceptance. Complete inhibition of reproduction was not achieved and the frequency of application was considered impracticable for a routine measure of rodent control.

Investigations to be reported in detail elsewhere have demonstrated that the 3-cyclopentyl ether of 17 α -hexa-1',3'-diynyl oestra-1,3,5(10)-trien-17 β -ol (BDH 10131) possesses a considerably longer duration of action than either mestranol or quinoestrol in the laboratory rat. The effects of a single dose lasted from a few days to more than six months, depending on the amount given. The potential of this compound for controlling the reproduction of wild *Rattus norvegicus* was accordingly examined in a preliminary pen trial in which bait was presented on three occasions at intervals of 8 weeks to a colony consisting of two males and six females of different ages. The bait consisted of 90% pinhead oatmeal, 5% whole-meal flour and 5% corn oil, with 0.015% w/w BDH 10131 added. Each 2 d period of baiting was preceded by 2 d of pre-baiting with a similar mixture from which the compound was omitted. Powdered SG 41 diet was provided *ad libitum* at all other times. A control colony was treated similarly but not given BDH 10131. Each colony was housed in a steel-sided enclosure approximately 9 m² in floor area and containing several wooden nesting boxes which were used to trap the animals, usually at intervals of 4 weeks, for population counts. The temperature was maintained at not less than 22° C and artificial lighting was provided from 06.00 to 21.00 h daily.

Food consumption decreased whenever BDH 10131 was presented, in spite of prebaiting to accustom the animals to the new food, but there was no persistent aversion to the bait similar to that reported with mestranol. The amounts of oestrogen ingested on successive applications of the bait were 5.4, 9.5 and 9.2 mg kg⁻¹, respectively, assuming that the number of animals was given by the most recent census and that the average body weight was 200 g. The population counts (Fig. 1) revealed a gradual decline in the size of the treated

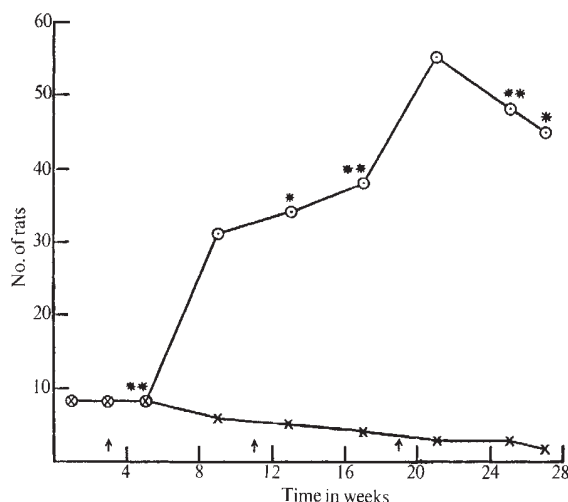


Fig. 1 Population counts in two pens each initially containing two male and six female wild rats (*Rattus norvegicus*). Bait containing 0.015% BDH 10131 was placed in one pen ($\times$ — $\times$) for periods of 2 d at 8 week intervals, as indicated by the arrows. A similar oatmeal : flour : corn oil mixture, but with no added BDH 10131, was supplied in the control pen ($\circ$ — $\circ$). Asterisks denote the presence of litters additional to the numbers of animals counted.

colony from the original eight to only two when the experiment was terminated after 26 weeks. Both survivors were males with gonadal atrophy. In contrast, the control colony increased steadily to a maximum of fifty-five rats at 21 weeks, only slightly less being maintained thereafter. Bimonthly exposure to the bait containing oestrogen thus completely prevented

reproduction throughout a 6 month period and seemed also to reduce the expected life-span of the female rats.

Although bait shyness did not seem to present a problem, a second pen trial was undertaken to determine the duration of the antifertility effect when groups of wild rats were exposed to the bait on a single occasion only. In this experiment, the animals were also given an alternative choice of food at the time of baiting as differences in acceptability might reduce the amount of bait consumed. Five pens similar to those used previously were each stocked with one adult and two pubertal males, plus two adult and four pubertal females. After an acclimatization period during which powdered SG 41 diet and water were provided *ad libitum*, one tray of diet in each pen was replaced by a tray containing the prebait used previously. Seven days later, bait containing either 0.002%, 0.01%, 0.05% or 0.25% w/w BDH 10131 was substituted for the prebait in all except the control pen. The bait was removed after 2 d and all pens were then provided with SG 41 diet as the sole food source for the remainder of the experiment.

The appetites of all groups were similar during the pre-baiting period, the food consumption of individual treatment groups ranging from 90% to 117% of that of the control group. There was a marked preference for prebait rather than SG 41 diet, the consumption of prebait during the 7 d period accounting for 75–87% of the total food consumption. Following the addition of BDH 10131 to the prebait mixture there was a marked reduction in the intake without any compensating increase in the amount of SG 41 diet consumed, thus suggesting some loss of appetite in addition to an aversion to the bait. The oestrogen intakes still proved sufficient to cause a dose-related period of infertility as shown by the population counts summarized in Table 1. The groups taking 3.2 mg kg⁻¹ and 16 mg kg⁻¹ showed no increase in number until 15 and 19 weeks after baiting, respectively, and even with an average

Table 1 Population Counts at Various Intervals after Application of Baits Containing Varying Concentrations of BDH 10131 for Two Days

Concentration of BDH 10131 in bait (%)	Total intake of BDH 10131 (mg kg ⁻¹)	-1	7	11	15	19	23	27	31	35	39	43	47	52
Total No. of rats present														
—	—	7	17	20*	24*	28*	37*	32	38*	42*		54*		
0.002	1.1	8	5**	21*	19*	27	28*	38*	38*	44***		73*		
0.01	3.2	8	7	7	10	8*	17	18	16**	24***	27**	50***		
0.05	16.0	7	7	7	7	10	11	11	23	24	30*	39**		
0.25	80.0	9	8	7	7	7	7	5	4	4	4	3	3	3

Each group initially comprised three male and six female wild rats (*Rattus norvegicus*), but fighting and cannibalism caused losses from some groups before baiting.

* No. of litters not included in total.

Table 2 Numbers of Rats Trapped at Two Refuse Tips Infested with Wild Rats

Trapping period	No. of trap-nights	No. of rats caught	Control tip No. of rats pregnant	No. weighing < 200 g	No. of trap-nights	Treated tip No. of rats caught	No. of rats pregnant	No. weighing < 200 g
Pre-treatment	144	27	4	16	240	60	6	14
Post-treatment interval								
4 weeks	216	58	7	35	240	47	0*	18
8 weeks	216	50	4	24	240	66	0†	29
12 weeks	216	50	4	31	240	28	0	2
16 weeks	216	37	1	23	264	44	0	0
20 weeks	216	53	2	15	264	24	0	1
26 weeks	240	58	4	18	264	14	2	0
32 weeks					264	11	1	0
38 weeks					264	11	0	0
46 weeks					264	7	2	0
56 weeks					264	0	0	0

Bait containing 0.05% BDH 10131 was present at one tip for 6 d, the other tip remained untreated.

* Two females with resorbing foetuses. † Three females with resorbing foetuses.

Table 3 Effect of BDH 10131 on the Pigeon

Experiment	Concentration of BDH 10131 in diet (%)	Total intake of BDH 10131 (mg kg ⁻¹)	No. of weeks post treatment						
			4	8	12	16	20	24	28
Cumulative total of eggs laid (No. subsequently hatched)									
1	—*	—	12 (7)	26 (14)	36 (24)	46 (33)	58 (39)	68 (48)	76 (56)
	0.0125	9.7	10 (6)	17 (11)	25 (15)	36 (22)	48 (28)	62 (39)	69 (41)
	0.04	19.6	5 (0)	11 (4)	21 (13)	34 (24)	40 (28)	54 (35)	60 (39)
	0.125*	72.1	0 (0)	4 (1)	8 (5)	18 (11)	22 (15)	27 (19)	35 (25)
2	—	—	16 (14)	30 (26)	46 (41)	52 (46)	64 (57)	75 (67)	81 (69)
	0.4	125	1 (0)	5 (0)	10 (0)	16 (2)	24 (2)	36 (12)	42 (13)
	0.8	231	2 (0)	2 (0)	4 (1)	8 (3)	12 (7)	15 (9)	19 (13)

Cumulative totals of eggs laid and subsequently hatched, recorded at 4-week intervals following the exposure of groups of pigeons (*Columba livia*; eight males and eight females) to varying concentrations of BDH 10131 for 2 d.

* 1 hen removed soon after treatment.

intake of only 1 mg kg⁻¹ there was some initial impairment of reproduction. The effect was particularly marked following the ingestion of 80 mg kg⁻¹, complete inhibition of reproduction then persisting until the experiment was terminated 52 weeks after baiting when only two males and one female remained. It would again seem that there was some reduction in the expected life-span of the animals, as in the original pen trial.

To determine whether the compound would be similarly effective under field conditions where migratory behaviour and differences in feeding habits might present additional complications, the reproductive performance of a rat population infesting a refuse tip was investigated following exposure to BDH 10131. The rats, numbering some 500–1,000, were treated by first prebaiting for 3 weeks with a mixture of 85% pinhead oatmeal, 5% wholemeal flour, 5% lactose and 5% corn oil, and then baiting for 6 d with a similar mixture containing 0.05% BDH 10131. Both prebait and treated bait were replenished as necessary to ensure maximum intakes and the consumption of treated bait over the 6 d period corresponded to approximately 60% of the amount taken in the last 6 d of prebaiting (equivalent to 14.9 g of BDH 10131).

The rats were sampled by removal trapping immediately before prebaiting was started, and at 4–10 week intervals after treatment was completed. Trapping was also carried out at similar intervals at an untreated control tip and the results summarized in Table 2 show marked differences between the two sites. Pregnant animals were consistently trapped at the control tip but it was not until 26 weeks had elapsed that the first rats with viable foetuses were caught following treatment with BDH 10131. The prolonged infertility of the females at the treated tip was further indicated by the virtual absence of juvenile animals among the rats caught 12 weeks after treatment and throughout the remainder of the study but at least 30% of the animals caught at the control tip were regularly classed as juvenile on the basis of body weight. The failure to reproduce was also reflected by a progressive decline in the total number of rats caught at successive trappings at the treated tip and within 6 months of treatment it was less than 25% of the pretreatment figure. The decline continued until, after about 1 yr, the colony appeared to be virtually extinct. In contrast, the number of rats at the control tip appeared to remain relatively constant throughout the period of investigation, the numbers caught at each trapping showing comparatively minor variations when allowance was made for differences in the numbers of traps laid. Although the rate of decline may have been exaggerated by removal trapping, the results indicate that the size of a wild rat population is markedly reduced within the space of a few months when reproduction is inhibited by even a single application of bait containing BDH 10131.

Investigations concerning the possible use of chemosterilants for the control of birds such as the pigeon (*Columba livia*)^{6,7} have led to attempts to produce a long-acting form of

mestranol^{8,9}. The use of BDH 10131 was investigated using an assortment of racing, fantail and feral pigeons divided into groups each consisting of eight cocks and eight hens. These were housed in suitable enclosures, each approximately 10 m² and initially having wire mesh partitions separating the sexes. No form of artificial heating was provided and natural daylight was the main source of illumination. 'Hormoform' commercial pigeon diet, flint, and limestone grit, and drinking water were all provided *ad libitum*.

After acclimatization, the 'Hormoform' was replaced in all except a control pen by a similar diet containing various concentrations of BDH 10131 mixed with suitable quantities of lactose to aid dispersion. This treated diet was removed after two consecutive days and an untreated diet supplemented by maple peas was provided for the remainder of the experiment. The partitions separating the sexes were removed when the treated diet was withdrawn and the birds were allowed to pair, suitable nesting boxes being provided. The dates on which eggs were laid and subsequently hatched were recorded throughout the following 7 months.

Although there was a considerable reduction in food intake by both sexes, considerable quantities of BDH 10131 were ingested during the 2 d period, with a mean intake amounting to 72 mg kg⁻¹ in the case of the highest concentration used (0.125%). The treatment resulted in a dose-related reduction in the total number of eggs laid and a corresponding reduction in the numbers subsequently hatched (Table 3) with no clear evidence of any increase in the proportion of eggs which were infertile or were neglected by the hens. The effect of the highest concentration was particularly marked during the first 12 weeks of the study, both the total number of eggs laid, and the number hatched, then being less than 25% of the corresponding figures for the control group. The impairment of fertility was evidently diminishing towards the end of the 28 week observation period, and the compound seems to have a somewhat shorter duration of action in the pigeon than in the rat. The results of a second experiment, in which the birds were allowed to pair before exposure to the bait, confirm that greater and more prolonged effects can be achieved by increasing the concentration of BDH 10131 in the bait to 0.4 or 0.8%. These concentrations produced total oestrogen intakes of 125 and 231 mg kg⁻¹, respectively, during the 2 d exposure period and both reduced the total number of fertile eggs laid during the first 28 weeks following treatment to less than 20% of the number laid by a control group. The main difference between the two concentrations was the number of infertile eggs which were also laid.

The application of chemosterilants to the control of pests such as the rat has hitherto been hampered by the lack of sufficiently long-acting compounds. These experiments clearly demonstrate the considerable potential of BDH 10131. Its duration of action in the rat is such as to permit the laying of bait less frequently than may be necessary with acute poisons and anticoagulants, and, in some circumstances at

least, even a single application may prove sufficient to eliminate an infestation. Use of the compound is not necessarily limited to the rat and evidence has also been obtained which indicates that the fertility of birds such as the pigeon can be considerably lessened by BDH 10131. These results thus warrant a more extensive evaluation of the compound in the field to assess its full potential.

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- ¹ Howard, W. E., *Pest Control: Biological, Physical and Selected Chemical Methods* (edit. by Kilgar, W. W.), 343 (Academic Press, New York, 1967).
- ² Brooks, J. E., and Bowerman, A. M., *Soap Chem. Spec.*, **45**, 58 (1969).
- ³ Brooks, J. E., and Bowerman, A. M., *Soap Chem. Spec.*, **45**, 82 (1969).
- ⁴ Marsh, R. E., and Howard, W. E., *J. Wildl. Mgmt.*, **33**, 133 (1969).
- ⁵ Brooks, J. E., and Bowerman, A. M., *J. Wildl. Mgmt.*, **35**, 444 (1971).
- ⁶ Elder, W. H., *J. Wildl. Mgmt.*, **28**, 556 (1964).
- ⁷ Wofford, J. E., and Elder, W. H., *J. Wildl. Mgmt.*, **31**, 507 (1967).
- ⁸ Wentworth, B. C., *Nature*, **220**, 1243 (1968).
- ⁹ Sturtevant, J., *Toxicol. Appl. Pharmacol.*, **19**, 634 (1971).

San Miguel Sea Lion Virus Isolation, Preliminary Characterization and Relationship to Vesicular Exanthema of Swine Virus

BETWEEN 1932 and 1954 there were repeated outbreaks of vesicular exanthema of swine (VES) in Californian swine herds, but since 1956 no cases have occurred in the United States and this has been attributed to a federal law which prohibited the feeding of raw garbage to swine¹. The continuing importance of vesicular diseases of swine, however, is suggested by reports from Italy², Hong Kong³, Austria, Poland and Britain (personal communication from A. H. Dariri, Plum Island Animal Disease Laboratory, 1973) of a vesicular syndrome in swine that could not be ascribed to foot and mouth disease virus (FMDV).

Epizootiological investigations of the outbreaks in California led Madin to postulate that vesicular exanthema of swine virus (VESV) arose from an unknown natural reservoir⁴, from which the virus entered raw garbage and hence swine. In recent years there has been a high incidence of abortion in the California sea lion (*Zalophus c. californianus*⁵), and in 1972 we isolated a picornavirus indistinguishable from VESV from one aborting animal on San Miguel Island, California. The isolate was designated San Miguel sea lion virus (SMSV).

Between March 27 and June 17, 1972, we studied aborting sea lions on San Miguel. Of ten aborted females and their foetuses selected for sampling, half had aborted at approxi-

mately 60 d and the other half about 30 d before full term. Ten adult females and their full term offspring were studied as controls. Virus isolation was attempted by swabbing the nose, throat, rectum and pharyngeal tonsil of each animal and introducing the swabs into tubes of Vero cells, PK-15 cells, sea lion skin cells and dolphin skin cells, all maintained in Eagle's minimal essential medium (MEM) supplemented with foetal bovine serum, 200 U of penicillin, and 100 µg of streptomycin ml⁻¹. Inoculated cultures were held at an ambient temperature of about 22° C for up to 5 d during the collection period, and were then transported to the laboratory and incubated at 37° C. Each culture was examined daily and frozen at -70° C after 10 d, or sooner if viral cytopathology was observed. All negative samples were blind passaged at least three times in each cell line.

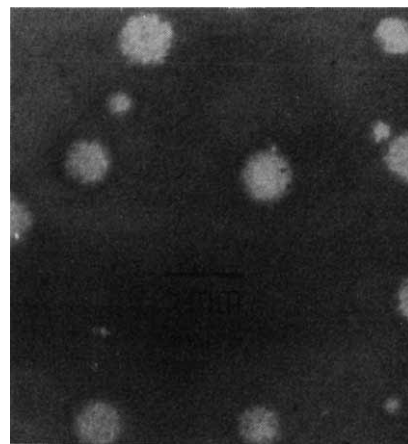


Fig. 1 SMSV plaques on Vero cell monolayers after 48 h incubation at 37° C are seen to vary from 1 to 4 mm in diameter.

The virus reported here was isolated on first passage in Vero cells from the rectal swab of an animal aborting 60 d before full term. By the second passage, the cytopathic effect (CPE) was evident within 24 h. Other susceptible cells showing CPE were porcine (PK-15), human primary (embryonic kidney) and lines of HeLa, Chang 1-5C-4 and foreskin. No CPE was observed with cell lines from marine mammals (dolphin skin, sea lion skin) or rodents (BHK 21, 1299). Plaques were readily produced in Vero cell monolayers overlaid with washed agar and incubated for 48 h at 37° C⁶. Plaque diameter varied from 1.0 to 4.0 mm (Fig. 1). Attempts to purify this isolate into homogeneous plaque populations using techniques described by Dulbecco⁶ were unsuccessful. Single step growth curves were determined using tubes of Vero cell monolayers inoculated with virus concentrations having a multiplicity of infection of 100. After 1 h of adsorption, monolayers were washed five times and replenished with MEM. At intervals of 2 h, duplicate infected cultures were frozen at -70° C, and later pooled and assayed for total virus content. Virus titres greater than 8 logs occurred after 8 h of incubation; titres began to decrease by 10 h after infection. Attempts to identify SMSV on the basis of serum neutralization tests were made by J. Schieble, California State Department of Public Health, and G. French, US Army, Fort Baker, California. All results were negative with antisera to polio 1-3, reovirus 2, coxsackie B₁-B₆, A₁, echo 1-9, 11-27, 29-33, enterovirus type 68 and rhinoviruses 1-89.

SMSV was shown to contain RNA by tritiated uridine incorporation⁷. Other properties such as ether sensitivity, instability at pH 2.7⁸, sensitivity to heat⁹, buoyant density, sedimentation coefficient (F. Schaffer, in preparation), and the detrimental rather than sparing effect of divalent cations

Table 1 Comparisons of VESV, SMSV, Italian Enterovirus and Hong Kong Vesicular Virus

	VESV	SMSV	Italy 1/66 ²	Hong Kong virus
Stability pH 4-5	pH 5-stable ¹⁴	Not tested	Stable ²	pH 4-stable ³
Stability pH 2.7-3	pH 3-labile ¹⁵	pH 2.7-labile	Not tested	Not tested
Ether resistance	Resistant ¹⁵	Resistant	Resistant ²	Not tested
Heat resistance 50° C	Labile ¹⁰	Labile	Labile ²	Labile ³
Stabilization by 1 M MgCl ₂ at 50° C	Not stabilized ¹⁰	Not stabilized	Stabilized ²	Stabilized ³
Sedimentation coefficient (S)	207S ¹⁵ 160-170S ¹⁴	180S	150S ²	Not tested
Buoyant density (g ml ⁻¹) in CsCl	1.36-1.38 g ml ⁻¹ ¹⁴	1.37 g ml ⁻¹	1.34 g ml ⁻¹ ²	1.34 g ml ⁻¹ ³
Size (nm)	35-40 ¹⁴	32	30-32 ²	28 ³
Morphology	Spherical with projections and dark areas ²	Spherical with projections and dark areas	Roughly spherical ²	Spherical
Susceptible hosts	Swine but not mice, guinea-pigs, hamsters, or rabbits ¹	Swine but not mice, guinea-pigs, hamsters, or rabbits	Swine and 1-d-old mice but not guinea-pigs, hamsters or rabbits ²	Swine; other comparable species not tested ³

(molar MgCl₂) in the presence of heat¹⁰ are the same for VESV and SMSV (Table 1).

Vero cells, fixed at 4° C in glutaraldehyde 12 h after virus inoculation, were examined by thin section electron microscopy. Intracytoplasmic viral lattice forms and viral particles in tubular configurations (Fig. 2) were observed. Such tubular configurations are characteristic of certain caliciviruses¹¹ such as VESV type H₅₄ (ref. 12). Individual virions were 32 nm in diameter. In addition to identical physicochemical properties and morphology, SMSV resembles VESV in its inability to cause disease in mice, rabbits, guinea-pigs and hamsters, as well as its capacity to produce lesions in swine¹. SMSV injected intradermally in the lip of two swine produced erosions with raised, moist coverings which spread by extension. These lesions were not typical vesicles but were compatible with those caused by less virulent strains of VESV such as F₅₅, G₅₅ and J₅₆ (ref. 1). Neither animal had a rise in temperature nor other clinical manifestations. Both were immediately destroyed without further testing because of the similarities of the lesions to those of VES.

Significantly, the serum of the aborted female yielding the SMSV isolate possessed a serum neutralizing activity in a titre

greater than 1:10. With the exception of two individuals who possessed no serum neutralizing activity whatsoever, the remaining seventeen, including all ten control animals, possessed neutralizing antibody activity ranging from 1:10 to 1:80. There were no essential differences in neutralizing titre between the ten aborting females and the ten control females. A virus isolate from the nasal cavity of the foetus freshly aborted by the female described above was, on initial examination, similar to, if not identical with, the mother's virus. Although it is well established that VESV¹ and other picornaviruses can cause abortion or reproductive disturbances of swine¹³ our data are insufficient to establish that SMSV is aetiologically involved in sea lion abortions. What is important is the finding of a marine mammal reservoir for a virus currently indistinguishable from vesicular exanthema of swine virus. This represents the first report of a virus from a marine mammal capable of producing disease in a terrestrial mammal.

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¹ Madin, S. H., *Disease of Swine* (edit. by Dunne, H. W.), third ed., 270 (Iowa State University Press, Ames, 1970).

² Nardelli, L., Lodetti, E., Gualandi, G. L., Burrows, R., Goodridge, D., Brown, F. O., and Cartwright, B., *Nature*, **219**, 1275 (1968).

³ Mowat, G. H., Darbyshire, J. H., and Huntly, F. J., *Vet. Rec.*, **90**, 618 (1972).

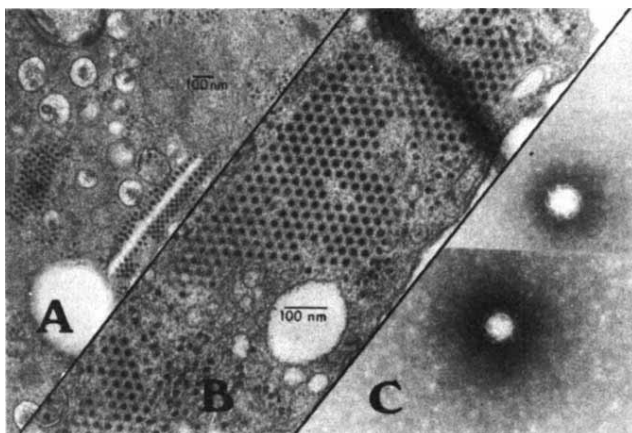


Fig. 2 Electron photomicrographs of Vero cells 12 h after infection reveals: (A) virus particles in tubular configuration similar to that seen with VESV type H₅₄ × 28,400; (B) a crystalline lattice that occurs in the cytoplasm × 57,600, and (C) a negative stained preparation of SMSV virus showing typical calicivirus morphology.

- ⁴ Odell, D. K., *Proc. Seventh Annual Conf. Biological Sonar and Diving Mammals*, 185 (1970).
- ⁵ Dubovi, E. J., and Akers, T. G., *Proc. Soc. Exp. Biol. Med.*, **139**, 123 (1971).
- ⁶ Dulbecco, R., and Vogt, M. J., *Exp. Med.*, **99**, 167 (1957).
- ⁷ Andrewes, C. H., and Horstmann, D. M., *J. Gen. Microbiol.*, **3**, 290 (1949).
- ⁸ Hamperian, V. V., Hilleman, M. R., and Ketler, A., *Proc. Soc. Exp. Biol. Med.*, **112**, 1040 (1963).
- ⁹ Wallis, L., and Melnick, J. L., *Virology*, **16**, 504 (1962).
- ¹⁰ Zee, Y. C., and Hackett, A. J., *Arch. Ges. Virusforsch.*, **20**, 473 (1967).
- ¹¹ Wildy, P., *Monograph in Virology*, **5**, 55 (1971).
- ¹² Zee, Y. C., Hackett, A. J., and Talen, L., *Virology*, **34**, 596 (1968).
- ¹³ Dunn, H. W., Gobble, J. L., Hokanson, J. F., Kradel, D. C., and Bubach, G. R., *Amer. J. Vet. Res.*, **26**, 1284 (1965).
- ¹⁴ Wawrzyniewicz, J., Sinale, J., and Brown, F., *Arch. Ges. Virusforsch.*, **25**, 337 (1968).
- ¹⁵ Oglesby, A. S., Schaffer, F. L., and Madin, S. H., *Virology*, **44**, 329 (1971).

Insecticidal Activity of Recent Bacterial Isolates and their Toxins against Mosquito Larvae

ALTHOUGH *Bacillus thuringiensis* is proving to be a useful pathogen against insect pests of field crops¹⁻³, there has been no equivalent bacterium that might be used in the control of insects, such as mosquitoes, that carry disease. Since 1966, however, the World Health Organization (WHO) has been seeking such a bacterium. Field workers in various parts of the world collect infected insects⁴ and send them to an International Reference Center (IRC) at Ohio State University. Here the causative agent of infection is investigated and material is distributed for research on potential control organisms. I have examined bacteria isolated from infected larval mosquitoes collected in Delhi, India (accession WHO/IRC No. 1321); those of the *Bacillus sphaericus* group had higher insecticidal activity than previously reported⁵, and those of the *Bacillus alvei-circulans* group had never before been implicated in disease of mosquito larvae. Either or both may eventually be useful in the biological control of mosquitoes.

Bacteria were isolated by plating the WHO/IRC material on brain heart infusion agar and growing it at 30° C for several days. Ninety-one isolates killed *Culex pipiens*, variety *quinquefasciatus* (the southern house mosquito): forty of them were identified as *B. alvei*, *B. circulans*, or intermediate strains^{1,6-8} and fifty-one belonged to the *B. sphaericus* group. The *B. alvei-circulans* group was grown on brain heart infusion broth at 30° C and bioassayed after 24 h. The *B. sphaericus* group was grown in a liquid synthetic medium and treated similarly². Insecticidal activity of this group was greater when grown in this medium than when ordinary laboratory broth was used.

The insecticidal activity of *B. alvei-circulans* was found in the supernatant. The soluble toxin was heat labile and destroyed at 60° C. In contrast, the insecticidal activity of *B. sphaericus* seems to be related to the cells themselves. The effectiveness of these isolates against the susceptible mosquito larvae may depend on infection rather than on any toxin.

The activity of some isolates is illustrated in Table 1. As the toxin-like material of *B. alvei-circulans* has not been isolated or definitively characterized, its insecticidal activity is expressed in terms of how far the original broth culture can be diluted and still kill 50% of the insect population. For *B. sphaericus*, results are expressed in terms of the number of cells ml⁻¹ of the test system capable of killing 50% of the test insect population. To compare this activity with that of *B. alvei-circulans*, the number of cells per ml is also expressed in terms of dilution: for example, *B. sphaericus*/II-1 needed

only 230 cells ml⁻¹ to give 50% death, which was equivalent to diluting the broth culture 1 to 4.4 × 10⁶. The insecticidal activities of two other *B. sphaericus* strains are included: *B. sphaericus*/K, isolated by Kellen *et al.*⁵, takes 10⁶ cells to kill 50% of the test insect population. Strain Q-s, derived from strain K, is more potent. As an indication of the order of magnitude of the insecticidal activity shown in Table 1, if *B. thuringiensis* was grown in an equivalent laboratory system with the appropriate medium and tested against its appropriate target insect, a dilution of the broth culture of 1/1,000 to 1/5,000 to kill 50% of the test population would be considered an extremely active culture and about as high as would be expected for *B. thuringiensis*.

Table 1 Insecticidal Activity of Broth Cultures of Several Bacteria that Attack Mosquito Larvae

Isolate	Insecticidal activity against <i>Culex pipiens</i> (causing 50% death of insect populations)
(A) Delhi isolates; soluble toxin producers	
<i>B. alvei</i> (aberrant)/III-3	1/1,000 dilution of original broth
<i>B. circulans/alvei</i> intermediate/VI-12	1/1,000 dilution of original broth
<i>B. alvei</i> /VII-6	1/1,000 dilution of original broth
(B) Delhi isolates; others	
<i>B. sphaericus</i> /II-1	230 cells/ml ⁻¹ or 1/4.4 million dilution of original broth
<i>B. sphaericus</i> /IV 8-B	2,400 cells ml ⁻¹ or 1/420,000 dilution of original broth
(C) From Kellen	
<i>B. sphaericus</i> /K	10 million cells ml ⁻¹ or 1/100 dilution of original broth
(D) Derived from strain K	
<i>B. sphaericus</i> /Q-s	50,000 cells ml ⁻¹ or 1/20,000 dilution of original broth

Cultures of all groups divided vegetatively for 5-8 h and then sporulation began, taking from 8 to 15 h to complete. If samples are taken during both the vegetative and sporulation cycles, the initiation of insecticidal activity can be identified. Both the soluble toxin of *B. alvei-circulans* and the active stage of *B. sphaericus* appear (insecticidal activity is detectable) after growth and vegetative division of the cells have ceased and the sporulation cycle has begun. They are apparently a product or result of the sporulation cycle.

The insecticidal activity as evidenced by death of the larvae took 5-7 d in the case of *B. alvei-circulans*. Insecticidal activity is usually recorded at 7 d, when larvae still capable of pupating will do so. In addition, once insecticidal activity reached a maximum in terms of samples taken during the vegetative and sporulation cycles, the activity did not decline.

B. thuringiensis insecticidal activity against Lepidoptera is related to a crystalline protein parasporal body (called delta toxin), formed when the spore is formed, and released when the spore is released^{9,10}. The bacterium can also produce a soluble heat stable exotoxin (beta exotoxin) active against Diptera. Production of a heat labile solution exotoxin by this organism has also been reported. Finally, several soluble toxins, lecithinases and such like, have been reported during vegetative growth of *B. thuringiensis*. The soluble toxin from *B. alvei-circulans* isolates seems to be different, however. It differs from the delta toxin in having no evident parasporal body, and from the beta toxin in not being heat stable: it is unlike the *B. thuringiensis* vegetative toxins because it is produced after vegetative growth. The only comparable toxin is the *B. thuringiensis* heat labile exotoxin. Unfortunately there has only been one report of this toxin^{9,10} and little is known about it.

In contrast the insecticidal activity of *B. sphaericus* peaked midway through sporulation and eventually decayed to one-third to one-tenth of its maximum value. In addition, insecti-

cidal activity manifests itself within 2 d and larval death is often seen within 12 h of the start of the bioassay. Thus although the insecticidal activity of *B. sphaericus* isolates seems to depend on an infection of susceptible larvae, some particulate toxin may be involved. Although cell numbers provide a convenient measure of relative activity, they may be irrelevant since the total population remains constant during sporulation even though activity decreases. The different insecticidal activities of the various *B. sphaericus* strains (of the order of 100,000 although (not shown here) the populations of the broth cultures are the same) suggest that infection of susceptible larvae does not involve a classical invasion by the bacterial cell and subsequent proliferation within the larvae as the prime mode of insecticidal activity. This is borne out by the early work of Kellen⁵, who when working with less active strains (see *B. sphaericus* K, Table 1) found that although there was extensive damage to the larval tissues before death, the bacteria did not break through the gut wall into the haemocoel until the larva was very close to death. The histopathology of the insecticidal activity of both the *B. alvei-circulans* and the *B. sphaericus* isolates is being studied by Dr Elizabeth Davidson at the University of Rochester, and should shed further light on the mode of action of the insecticidal activity of these bacteria.

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- ¹ Wolf, J., and Barker, A. N., *Identification Methods for Microbiologists*, Part B (edit. by Gibbs, B. M., and Shapton, D. A.), 93 (Academic Press, New York, 1968).
- ² Singer, S., Goodman, N. S., and Rogoff, M. H., *Ann. NY Acad. Sci.*, **139**, 16 (1966).
- ³ Burges, H. D., and Hussey, N. W., *Microbial Control of Insects and Mites*, 861 (Academic Press, New York, 1971).
- ⁴ Cantwell, G. E., and Laird, M., *J. Invert. Pathol.*, **8**, 442 (1966).
- ⁵ Kellen, W. R., Clark, T. B., Lindegren, J. E., Ho, B. C., Rogoff, M. H., and Singer, S., *J. Invert. Pathol.*, **7**, 442 (1965).
- ⁶ Smith, N. R., Gordon, R. E., and Clark, F. E., *Aerobic Spore-forming Bacteria* (Agric. Monograph No. 16, USDA), 47 (1952).
- ⁷ Knight, B. C., and Proom, H., *J. Gen. Microbiol.*, **4**, 508 (1950).
- ⁸ Proom, H., and Knight, B. C., *J. Gen. Microbiol.*, **13**, 474 (1955).
- ⁹ Heimpel, A. M., and Angus, T. A., *Bact. Rev.*, **24**, 266 (1960).
- ¹⁰ Anderson, R. F., and Rogoff, M. H., *Advances in Chemistry*, **53**, 65 (American Chemical Society, Washington DC, 1966).

Was Oddity Conspicuous in Prey Selection Experiments?

MUELLER¹ presented results of an extensive experiment designed to examine the effects of prey oddity, prey conspicuousness and specific searching image on selection of mice by two species of hawks. He concluded that oddity was more important than conspicuousness in prey selection. Other investigators have, however, demonstrated the effect of conspicuousness on prey selection²⁻⁵ when equal (no odd prey) numbers of two prey types (mice) were used. In addition, I found a significant positive correlation between effectiveness of selection by owls and degree of conspicuousness of the conspicuous prey (laboratory mice) in large pens with natural vegetation⁵. The contrast between the results of Mueller¹ and others²⁻⁵ led me to ques-

tion Mueller's experimental design for simultaneously testing selection of both odd and conspicuous prey by hawks.

As prey oddity is defined with respect to the numbers of individuals of each phenotype and prey conspicuousness with respect to prey and background coloration, it is necessary to recognize the background against which the predator perceives his prey before the effects of prey oddity and conspicuousness can be examined. Under Mueller's experimental conditions¹, (9 : 1 ratio of colour types of mice presented on pedestals close together and close to the hawk), the mice become a major part of the background against which the prey are seen as illustrated in Fig. 1. In these experiments, there was an odd prey on a background of nine non-odd prey plus the painted substrate. The painted substrate resembled the odd mouse in two experiments (Fig. 1A and C) and contrasted to the odd mouse in two experiments (Fig. 1B and D). Therefore, in this type of experiment the odd prey was conspicuous with respect to the "prey background" regardless of whether the "non-prey background" (painted substrate) resembled the odd or non-odd prey in coloration (see the odd spot that is conspicuous in each part of Fig. 1).

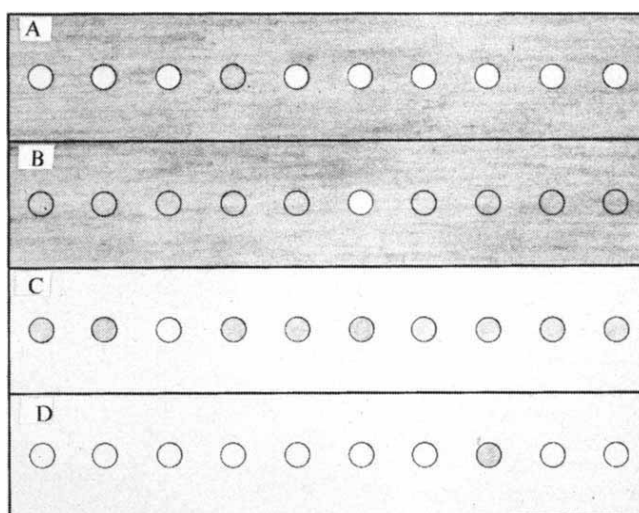


Fig. 1 Representation of selection experiments in which an odd prey of one colour and nine prey of a second colour are presented to the predator. In this type of experiment prey are placed in a small area and become a major part of the background. A, Odd prey matches dark substrate. B, Odd prey contrasts to dark substrate. C, Odd prey matches light substrate. D, Odd prey contrasts to light substrate.

Mueller's¹ results are in part due to selection of conspicuous prey against the "prey background". Thus, the importance of oddity and conspicuousness in prey selection cannot be determined because captures due to conspicuousness with respect to the "prey background" were included in the oddity category. For the same reason selection of odd pigeons by goshawks⁶ may be due to selection of conspicuous prey against the "prey background" (pigeon flock) rather than to oddity. Also, examination of Fig. 1 suggests that in experiments designed to test the effect of oddity on prey selection, the density of the prey must be low so that the coloration of the most common prey does not form a background against which the odd prey becomes conspicuous.

This communication was prepared while I was a postdoctoral fellow on an NIH training grant.

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- ¹ Mueller, H. C., *Nature*, **233**, 345 (1971).
² Dice, L. R., *Contrib. Lab. Vert. Biol.*, University of Michigan, **34**, 1 (1947).
³ Brown, L. N., *J. Mammal.*, **46**, 461 (1965).
⁴ Kaufman, D. W., *The Auk*, **90**, 204 (1973).
⁵ Kaufman, D. W., thesis, University of Georgia (1971).
⁶ Pielowski, Z., *Ekologia Polska* (A) **9**, 11 (1961).

Dr Mueller writes:

DR KAUFMAN'S analysis is naive and ignores information given in several of my publications and papers I have presented at meetings, at some of which I believe he was in the audience. My first report clearly indicates that I was aware of the ambiguity of Pielowski's² results which can be interpreted as the prey being conspicuous relative to the flock rather than evidence for selection for oddity. All of my experiments have been designed to segregate the effects of conspicuousness. My ten mice were on pedestals 10 cm square on an arc of a circle 2 m in radius³. The hawk was perched at the centre of the circle and 85 cm above the level of the pedestals. The pedestals were affixed to a board 122 × 244 cm, painted the same colour as the pedestals and those constituting the substrate. Measurements for four photographs taken with the camera at the hawk's perch showed that, on the average, the ten mice covered 1.6% of the substrate as viewed by the hawk. The mean distance between mice was 2.9 times their average width. I submit that the mice formed an inconsequential portion of the substrate and that Kaufman's Fig 1 is a simplistic and misleading portrayal of my experimental design.

My most recent presentation⁴ shows clearly that hawks select odd mice when confronted with a choice between only two mice, an experimental design which renders Kaufman's criticism completely invalid. Each hawk was presented with a single mouse of a given colour for ten consecutive captures, and then offered a choice between a grey and a white mouse on the eleventh trial. Hawks exhibited a very strong tendency to select the mouse which was odd in terms of the bird's prior experience. Full publication of these, and other, results of my experiments will come in due course.

The differences between my experiments and those cited by Kaufman are many. The lack of obstructions or "cover" for my mice would seem important, as would the fact that I carefully controlled essentially all but the experimental variables. Basically, the argument should be whether the results of carefully controlled laboratory experiments are applicable to nature, and whether experiments and observations under more natural, and variable, conditions can be performed with sufficient accuracy to reveal all of the factors influencing a behaviour.

I thank the US National Science Foundation for support of my work.

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- ¹ Mueller, H. C., *Nature*, **217**, 92 (1968).
² Pielowski, Z., *Ekologia Polska* (A), **9**, 11 (1961).
³ Mueller, H. C., *Nature*, **233**, 345 (1971).
⁴ Mueller, H. C., *Amer. Zool.*, **12**, 656 (1972).

New Method for Measuring the Flight Altitude of Birds

WE have measured the flight altitudes of homing pigeons and swifts using a new type of altimeter carried by the birds. The altimeter is based on the fact that the range in air of α -particles from a radioactive substance is inversely propor-

tional to the density of the air and that air density decreases in a regular way with altitude. The altimeter used with the pigeons consists of a lucite tube with outer dimensions of 50 × 8 × 5 mm³ (Fig. 1). The α -particle source (²¹⁰Po) is protected by a thin plastic film and inserted through slits in one end of the tube. The detectors are sheets of cellulose acetate plastic placed in the two 45° slits in the other end of the tube, making it possible to measure the maximum range and range distribution of the α -particles. The altimeter weighs about 1.0 g and is open at both ends so that the air may pass through freely without significant heating. Experiments performed by the Aeronautical Research Institute of Sweden have shown that pressures inside and outside the tube are nearly equal, representing an error in altitude of only 10 m (too high). There is no systematic error in the air density determinations. The precision in a determination of the maximum range depends on the source strength and the duration of the flight. The error amounts to about 0.2–0.4 mm, which corresponds to an error of about 100–200 m in flight altitude in the homing pigeon experiments.

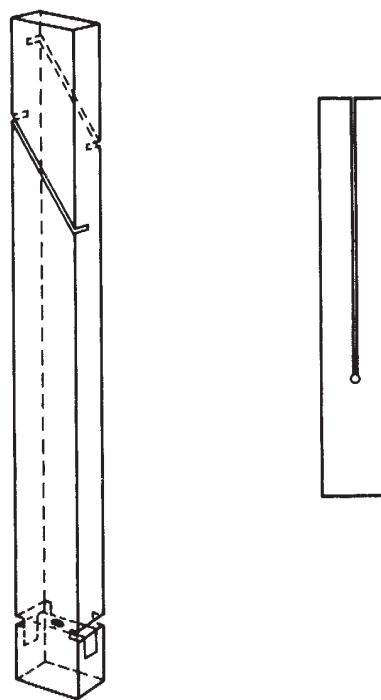


Fig. 1 Schematic drawing of the bird altimeter used in the homing pigeon experiments.

The altimeter is pasted to the bird's back immediately before the bird is released and removed after the flight. Figure 2 shows a record of a pigeon's flight. The density of α -particle marks on the plastic strip is plotted as a function of the distance from the radioactive source. The relation between extrapolated α -particle range and altitude shown in Fig. 2b is based on meteorological data for the flight day.

Five experiments with homing pigeons were conducted. Three pigeons were flown repeatedly thus making possible studies on the flying pattern of individuals. Table 1 gives the maximum flight altitudes together with some meteorological data. A comparison of the altitudes shows that different pigeons have different flying habits but every pigeon seems to have a remarkable habitual flying pattern. The data indicate that a mechanism exists which determines each pigeon's flying altitude. There is no indication that

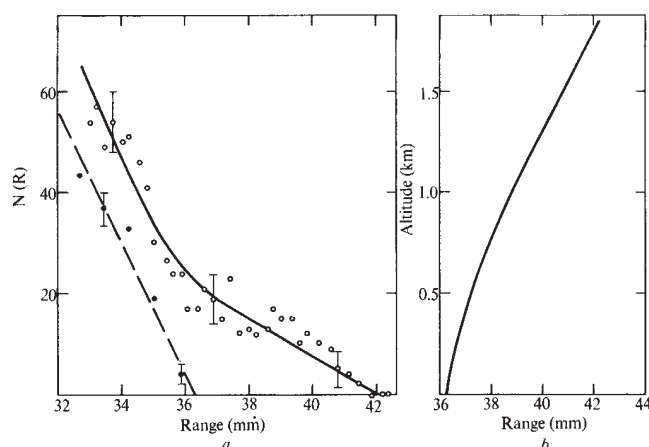


Fig. 2 The registration of a homing pigeon flight (homing pigeon 118 1972-05-08). *a*, The number of α -particle marks $N(R)$ is plotted against the distance from the source. The dashed curve represents a laboratory calibration. *b*, The extrapolated range of α -particles against altitude.

temperature determines the flight height. More experiments are needed to decide if the pigeons fly at a constant altitude above the ground, at a constant pressure, or at a level of constant pressure difference relative to the starting point.

Table 1 The Results of the Homing Pigeon Experiments

Date and distance NNE Lund	Pigeon No.	Flying time (min)	Maximum altitude (m above sea level)	Temperature and pressure at maximum altitude (° C) (mb)
25.4.1971	83	35	300	+ 3 970
~ 10 km	97	49	750	- 1 920
	118	35	1,600	- 9 825
8.5.1971	83	58	450	+14 970
~ 40 km	97	50	700	+11 945
	118	40	1,700	+ 3 840
9.5.1971	83	46	350	+16 985
~ 35 km	97	45	700	+12 945
	118	38	1,350	+ 8 875
6.6.1971	83	255	100	+20 1,005
~ 300 km	97	222	—	—
	118	218	1,550	+ 7 840

The probable errors are: in altitude, 100–150 m; in temperature, 1–2° C; in pressure, 10–15 mb.

Field work with swifts was carried out at Kvismare bird station (15° E, 59° N). The altimeters were altered to 20 mm (0.5 g). The α -particle ranges were reduced by a thin absorber and the radioactive sources chosen were strong enough for the flight times to be measured. Four swifts were found to fly at between 1,400–2,000 m, five at between 2,000–3,000 m and seven at between 3,000–3,600 m. As an example of flight times, one swift spent 5.5–8.0, 1.5–2.0 and 18–19 h at an altitude of 2,500 m (± 100 m), 1,400 m (± 100 m) and on the ground respectively.

Extended results and discussions will be published elsewhere.

We thank Drs G. Rudebeck and S. Ulfstrand; Professor Dirac for his communication; our pigeon expert, R. Jönsson; our collaborators in the swift experiments, R. Gyllin, L. Gotborn and B. Thyselius; the meteorologist, W. Olsson, and the Aeronautical Research Institution of Sweden for their

help. We also thank the Swedish Natural Science Research Council for financial support.

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Versatile Interactive Graphics Display System for Molecular Modelling by Computer

THE intricacies of constructing molecular models of macromolecules based on coordinates determined by X-ray crystallography has become a major task in itself, particularly now that a large number of protein structures has been determined. The combination of a computer and a graphics display terminal can be a powerful tool in studies of macromolecules. Several computer-based systems for the display of molecular structures have been described which differ greatly¹⁻⁵ and it was recently suggested that smaller computers can provide adequate capability⁶.

We would like to describe the interactive molecular display and modelling system (XRAY) we have developed at the National Institutes of Health. Any view in three-dimensions of a molecule, or any section of it in close-up, can be displayed, with the option of deleting any groups as a class or in any combination. A simple stick or a ball and stick (ORTEP)⁷ plot showing a stereoscopic view of the chosen section of the structure can then be generated. This capability represents an original improvement over previously described molecular display systems, in which the output has generally been by direct photograph of the screen. In addition the system we describe has been designed for the use of molecular biologists and biochemists and is both easy to use and versatile.

In such a system much depends on the particular combination of computer and graphics terminal used, and the computer programs written for them. The systems described so far tend to fall into two categories: those which use an expensive display device connected to a large dedicated computer¹⁻³, and those which use a less expensive graphics terminal with a dedicated mini-computer⁴⁻⁶. While the former are essentially unique systems which cannot be readily duplicated, the latter sacrifice capability for ubiquity. The system at NIH combines some of the advantages of both types. The hardware combination is based on a time-sharing DEC PDP-10 computer with two graphics display systems, an ADAGE AGT-30 graphics display computer and a DEC-340 graphics terminal. The versatility of the program package for molecular display is demonstrated by the fact that both these types of graphics terminals can be used. This results from the fact that all program choices (display and manipulation) are available to any remote graphics terminal over ordinary telephone lines in the interactive time-sharing mode. In addition, the ADAGE unit allows hardware-control by means of dials for rotation and manipulation. Details of the use of the system are described elsewhere⁸.

The input consists of Cartesian coordinates, usually derived from X-ray crystallographic studies, although the

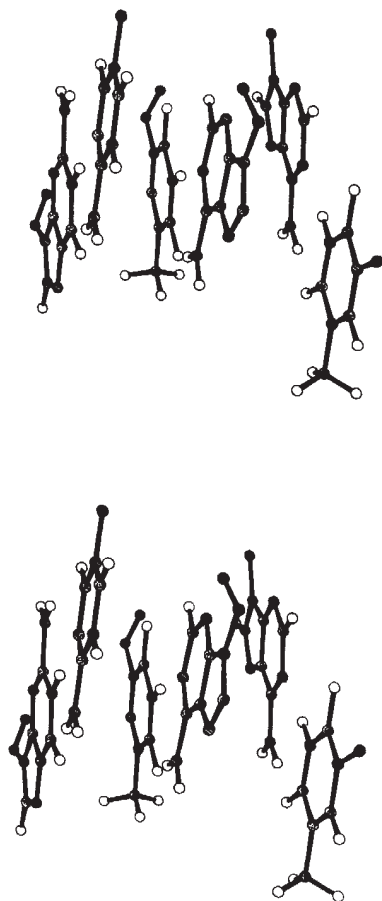


Fig. 1 Close-up stereoscopic view of three base pairs of DNA using the refined coordinates of the Watson-Crick double helical model⁹. This is a CALCOMP plot generated by the XRAY system using the ORTEP program⁷. The atom types drawn in are: open circles, H; striped, N; dotted, C; full circles, C, of sugar backbone.

coordinates can be generated from standard bond angles and bond distances. Up to 2,000 atoms in a single file (molecule) can be displayed, although this can be extended upwards. To produce bonds a table of connexions between atoms is set up by specifying a range of distances within which two atoms are to be connected, for example, for C-C bonds the distances generally used are 1.1–1.6 Å. Alternatively, algorithms are used to connect rapidly the long chain of atoms forming the protein backbone, as well as the residue side chains, based on their distinct branching properties.

The three-dimensional molecular structure can be displayed, and total rotation carried out about three orthogonal axes. A close-up view centred on any atom in the molecule can be obtained, viewed within a cube or a sphere of chosen dimensions. Interatomic distances and angles may be measured by specifying two or three atoms respectively. At any time specified groups, or combinations of groups, within the molecule, may be selected from the file (either in the full or "clipped" section) and can be saved as a separate file on disk. Examples of stereoscopic printout of such close-up, rotated sections are shown in Figs 1 and 2. These are ideal for instructional purposes, as are several films of specific proteins which have been made with the system.

In addition to the more effective visualization of macromolecules it is possible to use the manipulative capabilities of the system to rotate about bonds. As an example of this type of application not previously described the active

site of ribonuclease-S was chosen. There is some ambiguity regarding the positioning of the side chain of histidine-119 in the X-ray density contours and in fact several positions are possible^{10,12}. Figure 3 shows a structure in which the histidine-119 imidazole side-chain has been rotated about the C_α-C_β bond, and in which atom-atom contacts within a chosen distance are shown. Such an approach should aid in refining the results of X-ray crystallographic studies in the light of structural information available from other spectroscopic studies¹¹.

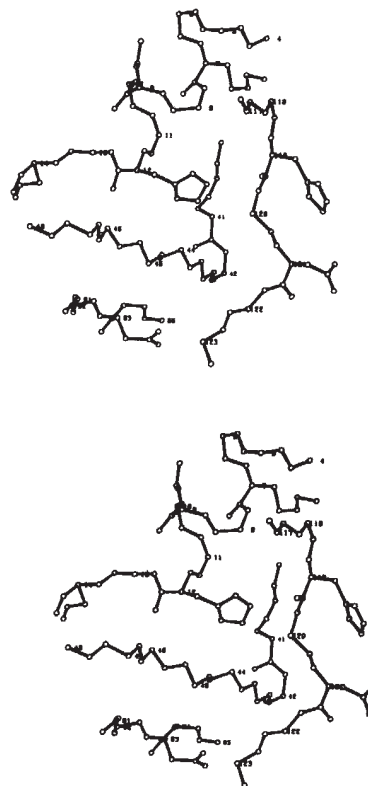


Fig. 2 View of the active site region of RNase-S (generated as in Fig. 1) showing only the charged amino acid side chains within a sphere of 12 Å diameter centred on the imidazole residue of histidine-12. This has been rotated in the XRAY system to minimize overlap in the image and is based on the refined coordinates of H. Wyckoff *et al.*¹⁰. The proximity of the imidazole residue of histidine-119 and the carboxyl group of aspartic-121 is noted¹¹. No hydrogen atoms are shown in this figure.

Several potentially significant aspects of this system are being developed. The X-ray coordinates used to put structures into the system are usually derived from the optical comparison of a hand-built model with the X-ray density contours¹³. As these contours are derived by computer calculation, and the final atom coordinates are refined by computer (ref. 14 and references therein) it would seem rational to carry out the model building process by computer as well. At present we are able to input a peptide of any sequence, but with a linear structure, in three dimensions by using the three-letter amino acid code names (program written by J. Kiefer). We can manipulate this to form helices. Planes of X-ray density contours can also be viewed, and superimposed upon any structure. The combination can, of course, be rotated in space, and any "slice" can be viewed automatically by working through

any segment of the molecule one plane at a time. This procedure is being used to "refine" coordinates of γ -chymotrypsin that were measured from a wire model constructed by Gerson Cohen, Jane Richardson, Enid Silverton and David Davies before the system was designed. To avoid having to build a whole molecule in this way, the structure of a known analogue may be used, altered accordingly. Using Diamond's model building program (ref. 14 and references therein), the results for the original and refined coordinates can be compared directly. This enables a quick comparison of the two images which was previously both difficult and inaccurate. Apart from manipulation of the chain, selective replacements of any amino acid side chain for any other side chain may be made at any point in the sequence.

Another adaptation of the system includes the calculation of the energy state of a given conformation (of a section with up to 35 atoms) using the CNDO/INDO program¹⁵. The "docking" of a small molecule into the

binding site of a macromolecule, together with the capability of energy minimization, may eventually lead to such computer-based systems becoming viable tools in molecular studies.

We thank Arnold W. Pratt and William Raub for support and encouragement, H. Wyckoff for allowing us to use the refined coordinates of RNase-S, and L. Katz for providing the refined DNA coordinates.

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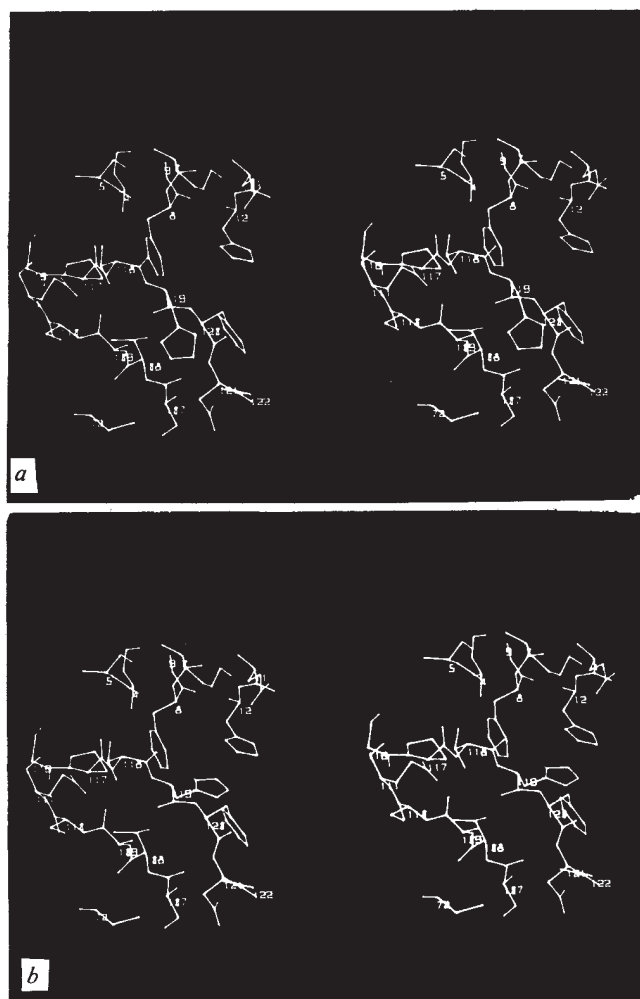


Fig. 3 Cubic sections (12 Å side) of the active site region of RNase-S centred on the C_{α} atom of histidine-119. Atomic contacts within 3.4 Å of the histidine-119 imidazole side chain atoms are shown by fine lines. This is the distance for closest atomic contact between the residues of histidine-119 and aspartic-121 (ref. 11), and is very close to the van der Waals distance (including the hydrogen atoms which are absent from the structure). In (b) the imidazole residue of histidine-119 has been rotated about its C_{α} - C_{β} bond to appear as close as possible to the imidazole residue of histidine-12. The lack of contact within this distance of the imidazole residues of histidines-12 and 119 is emphasized, since this has a bearing on the mechanism of action of the enzyme¹². These figures are polaroid prints taken directly from the display screen of the ADAGE unit.

- ¹ Levinthal, C., *Sci. Amer.*, **214**, 42 (1966).
- ² Katz, L., and Levinthal, C., in *Computer Graphics in Medical Research and Hospital Administration* (edit. by Parslow, R. D., and Green, R. E.), 56 (Plenum Press, New York, 1971).
- ³ Barry, C. D., Ellis, R. A., Graesser, S., and Marshall, G. R., in *Pertinent Concepts in Computer Graphics* (edit. by Faiman, M., and Nieuergelt, J.), 104 (University of Illinois Press, Urbana, 1969).
- ⁴ Perkins, W. J., Piper, E. A., Tattan, F. G., and White, J. G., *Comput. Biomed. Res.*, **4**, 249 (1971).
- ⁵ Barry, C. D., North, A. C. T., Glasel, J. A., Williams, R. J. P., and Xavier, A. V., *Nature*, **232**, 236 (1971).
- ⁶ Meyer, E. F., *Nature*, **232**, 255 (1971).
- ⁷ Johnson, C. K., ORTEP (ORNL-3794, 1965).
- ⁸ Feldman, R. J., *XRAY System Manual*, DCRT, NIH (in preparation).
- ⁹ Arnott, S., Dover, S. D., and Wonacott, A. J., *Acta Cryst.*, **B25**, 2192 (1969).
- ¹⁰ Wyckoff, H. W., Tsernoglou, D., Hanson, A. W., Know, J. R., Lee, B., and Richards, F. M., *J. Biol. Chem.*, **245**, 305 (1970).
- ¹¹ Schechter, A. N., Sachs, D. M., Heller, S. R., Shrager, R. I., and Cohen, J. S., *J. Mol. Biol.*, **71**, 39 (1972).
- ¹² Richards, F. M., and Wyckoff, H. W., in *The Enzymes* (edit. by Boyer, P. D.), **4**, second ed., 647 (Academic Press, New York, 1971).
- ¹³ Richards, F. M., *J. Mol. Biol.*, **37**, 225 (1968).
- ¹⁴ Diamond, R., *Acta Cryst.*, **A**, **27**, 436 (1971).
- ¹⁵ Quantum Chemistry Program Exchange, No. 141 (Chemistry Department, University of Indiana, Bloomington, Indiana).

Dislocations in Tobacco Mosaic Virus

WE would like to comment on the recent article with this title by Harris¹ in which he reproves our group for an alleged incorrect usage of the term "dislocation". In none of our papers do we write that the helical form of TMV is "dislocated or contains a dislocation", but rather that a disk will have to undergo a dislocation to be transformed into a short helical segment. As we have found that it is the disk which is the precursor for the assembly of the helix, it is natural to take the disk as the reference state for the subsequent rearrangements. In this case Dr Harris would presumably concede that our usage is quite normal, although he would prefer to describe the result with his newly-coined term "dispiration".

The main burden of his paper is to analyse in detail pictures such as those we had drawn^{2,3} to give some general feel for the process of assembly and the problem of incorporating the RNA. Harris takes the differences in detail in these pictures quite literally, but the main point in presenting a second picture was to include some perspective. It was not our intention to present the disk as a fixed entity which cannot break or lose some subunits during interaction with the RNA. In fact, recent experiments both here and in Berkeley⁴ have shown that a very rapid exchange can occur between individual

subunits in the A-protein and disk states both at equilibrium in the absence of RNA and also during the assembly reaction. Such a microequilibrium implies that the continuity of the disk structure cannot be assumed, and so the arguments for the gliding of a dislocation are not safe in this system.

It seems to us unjustified to make a direct transfer of concepts from the theory of crystal dislocations developed for metals and other strongly bonded crystals, composed of relatively rigid units, to the field of large biological molecules like TMV protein, which deform easily, which associate only weakly into aggregates of limited extent, and which are in rapid association-equilibrium in solution. For this reason we prefer to continue using simple, if somewhat imprecise, notions, of a "ruck" moving, for example, rather than to formulate detailed mechanisms as does Dr Harris.

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¹ Harris, W. F., *Nature*, **240**, 294 (1972).

² Butler, P. J. G., and Klug, A., *Nature New Biology*, **229**, 47 (1971).

³ Butler, P. J. G., *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 461 (1971).

⁴ Richards, K. E., and Williams, R. C., *Proc. US Nat. Acad. Sci.*, **69**, 1121 (1972).

Dislocations in Tobacco Mosaic Virus

I DO not allege that the way Butler and Klug use "dislocation" is incorrect. I merely point out that as crystal dislocations occur in tobacco mosaic virus (TMV) and may perform important functions, "confusion is bound to arise if the term dislocation continues to be used loosely". Confusion is even more likely when the term is used in the same context by the same group in both strict ("screw dislocation"¹) and loose (for example on p. 133 of ref. 2, "step dislocation at the end of the single helical rod") senses.

Butler and Klug deny my statement that members of their group refer to helical forms being dislocated or containing a dislocation. The second quotation above is sufficient to show their denial is false. I can supply a corroboratory quotation from every source I cite.

There is nothing wrong with taking the disk as the reference—I find a different reference more convenient. My reference is similar to Klug's radial projection³, which he and his coworkers use elsewhere but not here.

Just as Butler and Klug found it necessary or convenient to present detailed models "to give some general feel for the process", so have I. Without detailed models an explanation would be difficult if not impossible. What may appear to them "differences in detail" are in fact important differences: as I explain, one of their mechanisms converts the disk directly to a portion of a helix with the same number of subunits per turn as in the completed virion whereas the other requires an additional step.

My mechanisms, based on theirs, depend no more on "continuity of the disk" than do theirs. Just because subunits can be gained or lost in no way rules out dislocations. On the contrary, a very important mode of movement of dislocations requires the loss or gain of subunits⁴. It is similar to the mechanism of climb that I describe.

In their final paragraph Butler and Klug express an opinion which I am confident will be proved wrong. What can be

stated with certainty is that dislocations are present in many types of biological structures. The bibliography on dislocations in biological structures which I am compiling already contains more than a hundred references.

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¹ Butler, P. J. G., *Nature*, **233**, 25 (1971).

² Durham, A. C. H., thesis, University of Cambridge (1970).

³ Klug, A., Crick, F. H. C., and Wyckoff, H. W., *Acta Cryst.*, **11**, 199 (1958).

⁴ Harris, W. F., and Scriven, L. E., *Nature*, **228**, 827 (1970).

Low Mating Frequencies in an African Butterfly

DURING mating a male butterfly leaves a spermatophore in the bursa copulatrix of the female. One spermatophore results from each mating and so the number of times a female has mated can be determined by dissection of the bursa. Spermatophores can almost always be recognized even when old and collapsed and they persist throughout the life of the female¹ and in old and dried out museum specimens. In most butterflies the sex ratio is 1 to 1, wild-caught females are hardly ever virgins, most have mated at least once, and some have mated up to ten times.

The African butterfly, *Acraea encedon*, is exceptional in that in some areas, especially where the environment has been disturbed by man, there are populations which contain very few males. In Uganda there are populations where the males occur at frequencies of less than 4%, while elsewhere in Uganda and in some other parts of Africa the sex ratio is normal². The low sex ratios of some of these populations are known to have persisted for at least forty-five breeding generations. *Acraea encedon* is not parthenogenetic³ and to produce fertile eggs a female must mate, a process which lasts several hours. By breeding the butterflies on a large scale we have shown that there are two kinds of female, one producing females only, the other producing males and females in the expected 1 to 1 ratio, but so far we have not been able to find a way of distinguishing between the two kinds of female other than by breeding from them. We have postulated that the inheritance of the all-female broods is through a Y-linked gene (in butterflies the female is XY) causing meiotic drive in the Y chromosome, although we cannot entirely rule out the possibility of a cytoplasmic factor^{3,4}.

In populations where there are fewer than six males to every hundred females it is inconceivable that all females can be mated as males can mate only once a day and live for a maximum of two weeks. Females in predominantly female populations frequently lay infertile eggs on plants which are not the larval food and even on each other², and this alone suggests that many females remain unmated. Confirmation that many females do not mate has been obtained by dissecting samples of them for spermatophores from populations with various sex ratios (Table 1). From the spermatophore counts, most of the females in populations with few males are unmated and, of those that are mated, most have done so once only. In the six samples from four Uganda populations, less than a quarter of the females had mated, and in one of these samples all twenty-nine females were unmated. But in a sample from Sierra Leone where the sex ratio is nearly 16%, about 80% were mated, while in a small sample from a normal population in Sierra

Table 1 Mating Frequency of Females in Populations of *Acraea encedon* as Determined by Spermatophore Counts

Population	Approximate sex ratio of population*	Number of females examined	Number of spermatophores					% females with spermatophores	Mean number of spermatophores per female	
			0	1	2	3	4			
Uganda										
Lubya, October, 1971	0.025	67	62	5	—	—	—	7.5	0.08	
December, 1971		57	43	13	1	—	—	24.5	0.26	
Kololo, December, 1971	0.035	22	20	2	—	—	—	9.1	0.09	
Kawanda, December, 1971	0.039	29	29	—	—	—	—	0.0	0.00	
Budo, October, 1971	0.063	101	78	21	2	—	—	22.8	0.25	
December, 1971		58	48	9	1	—	—	17.2	0.19	
Sierra Leone										
Newton, October, 1972	0.156	123	25	86	10	1	1	79.8	0.92	
Gegbwema, June, 1972	0.500	6	—	2	2	2	—	100.0	2.00	

* In all populations there are slight fluctuations in sex ratio and the figures given are estimates based on continuous sampling in most cases.

Leone all had mated. Samples of other species of *Acraea* in which the sex ratio is normal show that hardly any females are unmated when collected and that most have mated more than once.

How, then, are the populations able to persist for generations when so few females are mated and where many contribute nothing to succeeding generations? The most likely mechanism is that normal females (those producing males and females) have a higher probability of mating than abnormal females (those producing females only). In all the predominantly female populations we have examined there have been slight fluctuations in the sex ratio and thus it would seem likely that any mating preference would be frequency-dependent such that normal females would be at their greatest mating advantage when their frequency is lowest. Attempts to obtain frequency-dependent matings in controlled conditions have so far been unsuccessful⁴, but this does not rule out the possibility that they occur in nature. It is also probable that the sequence of emergence of males and females in a mixed brood confers a frequency-dependent mating advantage on females from a normal brood, although most of such matings would be brother-sister.

Acraea encedon lays its eggs in large clusters and the caterpillars are gregarious until the last instar. Mortality of complete clusters of eggs or caterpillars is common, but those clusters that do survive seem to produce many adult butterflies. The males hatch a day or two before the females, but the developmental time of females from abnormal broods is nearer to that of males than to females from abnormal broods. Using the results from laboratory breeding, together with estimates of longevity obtained from capture-recapture experiments in the field, we have calculated that the maximum mating advantage that normal females can achieve is just over 1.4, and that this occurs when the normal females emerge one or two days after the males⁴. This compares quite well with the results from laboratory experiments in which normal females emerged on average 2.2 d after males. From this result we believe that normal females gain some mating advantage but this is not enough in itself to ensure the survival of the normal line.

The natural occurrence of low sex ratios in some populations of *A. encedon* is similar to the situation created when artificially sterilized males are released into a pest population in an attempt to control numbers. But our results suggest that one of the possible consequences of distorting the sex ratio artificially is that the population may become adjusted to the new circumstances, and will persist, although possibly in smaller numbers. It certainly seems that *A. encedon* can survive where males are in short supply and that females which contribute nothing to subsequent generations are continually produced.

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¹ Burns, J. M., *Proc. US Nat. Acad. Sci.*, **61**, 852 (1968).

² Owen, D. F., and Chanter, D. O., *J. Zool.*, **157**, 345 (1969).

³ Owen, D. F., *Nature*, **225**, 662 (1970).

⁴ Chanter, D. O., and Owen, D. F., *J. Zool.*, **166**, 363 (1972).

Tooth Chipping in the Australopithecines

ROBINSON¹⁻⁴ has suggested that the robust and gracile australopithecines had different diets; the robust forms were vegetarians, whereas the gracile forms were omnivores. Because he believed they occupied these different adaptive zones, Robinson separated the australopithecines generically: the robust types were called *Paranthropus* and the gracile types *Australopithecus*.

The incidence of tooth chipping in the australopithecines has been used both to support and to refute Robinson's dietary hypothesis. Robinson¹⁻⁴ claimed that the robust had more chipped teeth than the gracile australopithecines, and that this was evidence that the robust forms ate more grit-laden roots, bulbs and tubers. Tobias⁵ rejected this inference because the chips he examined were "similar in size, character and number per jaw" in both robust and gracile specimens. Moreover, the enamel chips Tobias saw were rather "large". These large chips, Tobias claimed, were produced by chewing on bones.

I evaluated these conflicting claims by examining the teeth of the South African early hominids under a low power binocular microscope for evidence of enamel chipping. An enamel chip is defined as a flake scar equal to or greater

than 0.1 mm. The surface features of rounding and scratching were used as criteria for sorting chips that were produced during an individual's lifetime (ante mortem chips) from those produced after death (post mortem chips). Unless otherwise qualified, the word chip in the following discussion means an ante mortem chip.

In a sample of 589 worn deciduous and permanent teeth, fifty chips were found spread among forty teeth representing an estimated twenty-nine individuals. Of the six chipped teeth that comprised Tobias's sample, only one (M^3 of Sts 53) can be accepted as an ante mortem chip; the other five, by my criteria, are post mortem chips.

The fifty chips were sorted into two groups according to size: forty-seven were small (0.1 to 2.8 mm) and are called micro-chips, three were large (approximately 4 mm) and are called macro-chips. I calculated three ratios of micro-chipping for the permanent teeth of Swartkrans, Sterkfontein (Type) and Makapansgat. These are as follows.

Chipped teeth = No. of chipped teeth / No. of worn teeth.

Chipped cheek teeth = No. of chipped cheek teeth / No. of worn cheek teeth.

Multiple chipped cheek teeth = No. of chipped cheek teeth with multiple chips / No. of chipped cheek teeth.

In addition, multiple chipping was expressed as the average number of chips per chipped cheek tooth for each of the three sites. Excluded from these calculations were the Kromdraai, Sterkfontein (Extension), Cooper's B and Taung teeth because the sample was too small. I also excluded the teeth in SK 15+SK 18a+SK 43 and the teeth in SK 45+SK 80/847/846b because I believe⁶, as do Robinson⁷ and others⁸, that these two individuals are not small robust australopithecines but early members of the genus *Homo*. Thus the comparison of tooth chipping was made between the robust australopithecines of Swartkrans and the gracile australopithecines of Sterkfontein (Type) and Makapansgat.

Table 1 Chipped Permanent Teeth from Sterkfontein, Swartkrans and Makapansgat (%)

Indices	Sterkfontein	Swartkrans	Makapansgat
(1) Chipped teeth	5.9	6.8	7.1
(2) Chipped cheek teeth	5.5	7.7	8.8
(3) Multiple chipped cheek teeth	14.2	12.9	0

The average number of chips on a cheek tooth was Swartkrans: 1.2, Sterkfontein (Type): 1.1 and Makapansgat: 1.0; and as Table 1 shows, the percentage values of the three indices differ to the order of 1, 2 and 3% among the sites and Swartkrans does not show the highest value for any of the three indices. This is not an appreciable difference in tooth chipping between robust and gracile australopithecines. Thus it seems unlikely that the robust australopithecines ate more "grit-laden roots, bulbs and tubers"⁴ than the gracile australopithecines. In conclusion, my observations on tooth chipping suggest that the robust and gracile australopithecines of South Africa did not differ substantially in the amount of grit in their diet.

I thank P. V. Tobias for advice in preparing the manuscript, C. K. Brain for access to the fossil hominids housed in the Transvaal Museum, and the Wenner-Gren Foundation for Anthropological Research and the University of the Witwatersrand for financial support.

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¹ Robinson, J. T., *Evolution*, **8**, 324 (1954).

² Robinson, J. T., *S. African J. Sci.*, **57**, 3 (1961).

³ Robinson, J. T., *Amer. J. Phys. Anthropol.*, **21**, 595 (1963).

⁴ Robinson, J. T., in *African Ecology and Human Evolution* (Aldine, Chicago, 1963).

⁵ Tobias, P. V., *Olduvai Gorge*, 2 (Cambridge University Press, Cambridge, 1967).

⁶ Wallace, J. A., thesis, Univ. Witwatersrand (1972).

⁷ Robinson, J. T., *Amer. J. Phys. Anthropol.*, **11**, 445 (1953).

⁸ Clarke, R. J., Howell, F. C., and Brain, C. K., *Nature*, **225**, 1219 (1970).

(continued from page 76)

natn. Acad. Sci. U.S.A. to biochemical knowledge would be considerably greater than the D/A index suggests. The index D/C provides a combined indication of compactness of information content (A/C) and proportion of significant papers of biochemical interest (D/A), and is thus a good index of value to the research biochemist. The index D/C may thus prove to be a better guide to selection of journals for subscription than position in the ranking list. When ranking is done on the basis of the value of D/C , *Biochem. biophys. Res. Commun.* occupies the first position and *Proc. natn. Acad. Sci. U.S.A.* the second.

Finally, I shall discuss my results in relation to Bradford's law of scattering⁴. The law states that "articles of interest to a specialist must occur not only in the periodicals specializing in his subject, but also, from time to time, in other periodicals which grow in number as the relation of their fields to that of his subject lessens and the number of articles on his subject in each periodical diminishes". The ranking list in Table 1 conforms closely to this law, the lower ranking titles in the list being less and less related to biochemistry as a discipline, and the number of citations of them being progressively fewer. The validity of Bradford's law is even more forcibly brought out when one takes into consideration the 453 titles which were excluded from the list because of the infrequency of their citations. Also, as would be expected from Bradford's law, the first eighteen journals in the list account for 73% of all citations counted. The results of the present study suggest a further extension of Bradford's law of scattering: that during phases of rapid and vigorous growth of knowledge in a scientific discipline, articles of interest to that discipline appear in increasing numbers in periodicals distant from that field. In other words, during phases of rapid growth of a scientific discipline, the small group of journals accounting for the larger part of the significant literature of the subject contain a relatively high proportion of unrelated journals.

I thank Dr A. Narayanaswami for his interest, encouragement and help and Professor R. N. Chakravarti, Director, Indian Institute of Experimental Medicine, Calcutta.

¹ Sengupta, I. N., *Int. Libr. Rev.*, **4**, 169 (1972).

² Henkle, H. N., *Med. Libr. Ass. Bull.*, **27**, 139 (1938).

³ Sengupta, I. N., *Int. Libr. Rev.*, **3**, 271 (1971).

⁴ Bradford, S. C., *Documentation* (Public Affairs Press, Washington, DC, 1950).

Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Modern Astronomy

Astronomy: Fundamentals and Frontiers. By Robert Jastrow and Malcom H. Thompson. Pp. xiv+404. (Wiley: New York and London, 1972.) £7.35.

THE authors' aim in writing this book was to provide the liberal arts student with a one-year course dealing with the central problems of twentieth century astronomy. It is hard to imagine how they could have performed their task more admirably. The sequence of the book is unusual. The stars, the galaxies and cosmology precede the sections on the solar system, the Earth and the evolution of life because the authors feel that an astro-physical background is required to discuss the evolution of the solar system. The professional astronomer is unlikely to advance his knowledge of the subject by reading this book but he will be given a brilliant lesson in exposition at an elementary level. Indeed I agree with the remark made by Bengt Stromgren in his foreword that the authors have made "a very significant contribution in the area of communication between scientist and the community".

An impressive feature of the writing is that the authors do not evade the difficulty of explaining the essential fundamental physics—for example of the nature of light or the production of spectra. The chapters on the evolution of the stars and the elements, in particular, are models of exposition at an elementary level. A typical example of the authors' style occurs when they pose the question of "How large must a cluster of atoms be before its own gravity is strong enough to hold it together permanently?" After some further exposition they quote the theoretical result as about 10^{57} atoms and then they continue in this manner: "This is a staggeringly large number. We have searched for a comparison that would make the meaning of the number 10^{57} clear, such as comparing 10^{57} with the numbers of grains of sand in all the beaches of the world; but even that seemingly uncountable number is only a mere 10^{25} . In fact, the number of neutrons and protons contained in the nuclei of all the atoms

of the entire Earth is only 10^{51} . The trouble is that no number on Earth can possibly match this number; it is a number that corresponds to the building blocks of objects the size of stars, not objects the size of planets." I can well imagine that the liberal arts student will be as stirred by such passages as I was to read in a later chapter an account of the most recent researches on the great plates into which the Earth's surface is divided. It will be hard to forget that the plate underlying the Pacific Ocean is scraping past a plate that includes most of the North American continent and that at the present rate of movement of 1.5 inches per year "Los Angeles will enter the suburbs of San Francisco in ten million years".

Only authors with a deep knowledge of the subject would be capable of writing this type of book. It is, therefore, the more surprising to find the occasional strange historical lapses. The discovery of the cosmological redshift is attributed to Slipher in 1926. Slipher discovered the redshifts from several nebulous objects in 1912; but they were believed to be galactic objects until Hubble in 1926 produced the observational evidence for their extragalactic nature. And surely neither Grote Reber's many admirers, nor Reber himself, would wish to claim that he discovered the radio galaxies in 1940. Although he observed enhanced radiation from the Cygnus region, astronomers had no idea of the significance of this for another 11 years. The sequence of observations which led to the discovery of the radio galaxy in Cygnus began in 1946 when J. S. Hey and his colleagues found that the intensity of the radio waves from Cygnus fluctuated. Their conclusion that the emission emanated from a source of small dimensions was confirmed by the interferometric measurements of J. G. Bolton and G. S. Stanley in 1947, but no further progress was made until the accurate positional measurement made by Graham Smith in 1950 enabled W. Baade and R. Minkowski to identify the object on plates taken with the 200 inch Palomar telescope in September 1951. It is a pity, too, that the authors give a poor and misleading account of the discovery

of the quasars. Although an account is given of Maarten Schmidt's identification of the spectral lines in 3C 273 in 1963, the sequence of British, American and Australian radio observations during the preceding years is a memorable event of 20th century astronomy and deserves appropriate treatment in a text of this nature.

The student will find his appreciation of the book enhanced by the excellent diagrams and many beautiful photographs of the sky. He will be stimulated to understand the text by the test questions which follow every chapter and he will no doubt be surprised to find that even in this most lavish of texts a number of omissions, errors and inconsistencies escaped the proof reader.

BERNARD LOVELL

Structure of Cells

Techniques of Biochemical and Biophysical Morphology. Edited by D. Glick and R. M. Rosenbaum. Pp. ix+280. (Wiley: New York and London, November 1972.) £6.25.

OF the writing of annual reviews there is no end. The editors of this new series state: "A pressing need has evolved for review volumes published at regular intervals, usually annually, to aid scientists in keeping up-to-date with developments in instrumentation, methodology and techniques of biochemical and biophysical morphology". Well, yes; we all make use of such volumes, quite often at the beginning of an investigation and frequently to check our sources of information at later stages. It is the definition of the subject matter that is the trouble. Quite a number of articles in this volume could with equal justification have appeared in another series, for example, the *International Review of Cytology*.

This volume contains eight articles on a wide range of subject matter. All contributions are supplemented by references to the literature and there are separate and useful author and subject indices. All these contributions seem to be competently written and to give good introductory guides to the present state of the subject. The first article on glycogen localization gives procedures

together with the appropriate chemical background and methods for overcoming the lack of specificity inherent in the procedures. Although a brief comparison of methods is given, no example of a single subject stained by each is presented.

In rapidly advancing subjects review articles suffer from lack of reference to the most recent contributions. Without blame to the authors, the chapter on microtubules and microfilaments is now rather out of date, but nevertheless collects together useful practical detail.

The article on "Partial Cell Irradiation with Conventional and Laser Sources" is not quite so general as the title would seem to indicate. "Conventional sources" is here synonymous with ultraviolet; no reference is made to proton beams, α -particle beams or the techniques of radioactively-tipped micro-needles. Within the narrower frame of reference of "ultraviolet and laser microbeams" the chapter is accurate and gives good cover of the field.

Overall, this series has started well; but it seems unlikely that more than one copy would be required within one school of biological science.

JOHN RANDALL

Geochemical Data

The Encyclopedia of Geochemistry and Environmental Sciences. Edited by Rhodes W. Fairbridge. Pp. xxi+1321. (Van Nostrand Reinhold: New York and London, October 1972.) £24.75.

THE science of geochemistry, as summarized by Professor Rankama and quoted in the preface, embraces the determination of the abundance of the chemical elements and atomic species in nature; the description of the distribution of the individual elements in the different spheres of the Earth and in minerals and rocks; and the elucidation of the laws governing the abundance and distribution of elements. It therefore includes the study of each naturally occurring element with respect to its concentration in the core, mantle and crust of the Earth and in extraterrestrial bodies, its behaviour during crystallization and differentiation in a magma and in metamorphism, its amount and mode of occurrence in mineral lattices, and its dispersion at the surface of the Earth.

This comprehensive reference work, which is Volume IVA of Van Nostrand Reinhold's *Encyclopedia of Earth Science* series, aims to cover all of the important facets of geochemistry implicit in the foregoing statement. It is also intended to embrace the study of the environmental sciences "because the chemical pollution of our planet's air and water is claiming the attention of so many of our geologists and chemists today". In spite of some good contribu-

tions on environmental aspects of earth sciences, this part of the work appears to have been added as an afterthought. There has been little attempt at integration in the bulk of the text, as demonstrated by the individual entries on the metals cadmium, lead and mercury, in which no mention is made of the important environmental studies which have taken place on these toxic elements in recent years.

Topics covered in the book are arranged in alphabetical order, and extensive cross-referencing, together with a twenty-five-page index in small but clear type, facilitates retrieval of information from the 20,000 or so entries. These were contributed by almost 240 specialist authors of whom about 190 were resident in the USA, but in spite of this preponderance of North American writers, one is not aware of any major geographical bias in the selection of data and examples of geochemical phenomena. The situation is less satisfactory in the matter of references. They are appended to every item in the book and though some are excellent, as for example those on the planetary abundance and distribution of elements, where numerous sources of original material are given, others, including that on spectrophotometry, list only a few rather dated texts usually North American in origin. In fairness to these authors it should be mentioned that the editor records apologies for not ensuring that certain articles were brought up to date at the last moment. However, this can scarcely be held to excuse the entry on chemical mineralogy which is of little more than historical interest and was apparently taken from a Reinhold publication of 1958.

It is inevitable that in a compilation such as this the standard of individual entries will vary and that there will be an imbalance in the treatment of topics. Hydrogeology and hydrology, for example, rightly occupy a large section (forty-eight pages) but estuarine hydrogeology, which must represent a major link in the geochemical and pollution cycles, is dealt with in barely four pages of text, while the large subject of mantle geochemistry occupies only six pages, including some very useful reference tables. On the whole, however, a satisfactory level has been achieved. Thus there is a concise and informative section on aluminium ore deposits which summarizes the definition, mineralogy and origin of bauxite, its present day formation and world distribution, in just over four pages. Certain other economic ore deposits are less satisfactorily dealt with, for example "Uranium: Economic Deposits", but since a separate volume (IVB) on mineralogy and economic geology is planned for the series, this is perhaps understandable.

Geochemical prospecting is also to be

covered by volume IVB. While the editor's problem over the proper location for this subject can be understood, the sampling, analytical and statistical methods evolved for geochemical prospecting and reconnaissance have application to environmental studies which merit their inclusion in this encyclopaedia. The problems of sampling and the statistical analyses and computer treatment of large volumes of data, which are basic to all geochemical studies, have perhaps been investigated most fully in recent years by applied geochemists engaged in the study of element distributions in superficial materials. They are mentioned briefly in the section on the planetary abundance and distribution of elements, but the geochemical reader looks for considerably more coverage of this subject in a book of this kind.

Similarly the situation with respect to "Analysis", which one would expect to find shortly after "Aluminium", is interesting. In an encyclopaedia on a subject in which analytical data are of fundamental importance, one might have expected the methods used in the acquisition of that data to be accorded some status. Not only is there no entry headed "Analysis", but the deficiency applies also to "Analytical Geochemistry", "Chemical Analysis" and "Geochemical Analysis". Recourse to the index reveals that a number of analytical techniques are listed separately, but the only one in which the whole field is surveyed appears to be that headed "Geochemistry: Testing for Elements". Contrary to the belief of the author of this section, EDTA is not an organic dye used for colorimetric analysis, and although one might sympathize with his sentiments, the petrographic microscope is not to be generally recommended for the qualitative analysis of elements. No mention is made of atomic absorption spectrophotometry in this section, although this is perhaps the fastest growth area of geochemical analysis, and other major techniques are omitted. In fact, atomic absorption methods are outlined elsewhere in an excellent, concise (four-page) and up-to-date entry under flame spectroscopy.

The standard of production of the book is high and the great majority of the many diagrams are of good quality. There is the occasional irritant, such as that on page 1115 where the labelling of a diagram does not coincide with the numbering of the components in the text, but misprints are few. Examples are that the sublimation temperature for molybdenum trioxide (page 753) is given as 1400° C instead of 795° and the end member of the U^{238} decay chain as Pb^{216} instead of Pb^{206} (page 994).

In spite of the inevitable shortcomings

inherent in the production of a book of this kind, it represents a valuable quick reference volume for any organization actively engaged in geochemistry. A vast quantity of basic geochemical data is contained in readily accessible form within its pages.

P. J. MOORE
D. OSTLE

Heat and Stability

Chemical Thermodynamics. Volume I. Senior Reporter M. L. McGlashan. (A Review of Recent Literature published up to December 1971.) Pp. xii+362. (The Chemical Society: London, February 1973.) £8.

APART from the introductory chapter contributed by Professor M. L. McGlashan, the whole of this book has been written by members of the thermodynamics group of the Division of Chemical Standards of the National Physical Laboratory. This has given it a cohesion which would otherwise have been difficult to achieve, and all those concerned may justifiably take a collective pride in the quality of the volume they have produced.

This first specialist report on chemical thermodynamics is concerned primarily with the determination and manipulation of thermodynamic quantities relating to chemical equilibria. This accounts for what would otherwise have seemed rather artificial criteria for limiting the scope of particular chapters. Thus, the admirable essay by Dr D. Ambrose on "Vapour Pressures" makes no mention of recent precise comparative studies of the vapour pressures of isotopic species. Low-temperature calorimetry is described in Dr J. F. Martin's lucid chapter on "The Heat Capacities of Organic Compounds", but because there is usually no need to make measurements on such compounds below 10 K to obtain reliable values for the standard entropy, there is nothing in this chapter on recently developed techniques for measuring heat capacities below 10 K or from, say, 400 K to 600 K. Nor will the reader generally find much discussion of what one contributor calls "the use of thermodynamics in the systematic understanding of chemistry", though the account by Drs S. G. Frankiss and J. H. S. Green on "Statistical Methods for Calculating Thermodynamic Functions" has a thorough survey of methods of this kind for determining potential barriers to intramolecular rotation, and Drs J. D. Cox and I. J. Lawrenson in their chapter on "The P, V, T Behaviour of Single Gases" discuss the connexion between second virial coefficients and intermolecular potential energy functions.

The value of this book lies chiefly (but by no means exclusively) in the wealth

of practical advice that it contains. Random examples of this are the detailed information given by Dr A. J. Head, writing on "Combustion and Reaction Calorimetry", on the methods used to contain samples for combustion, and the description of a flow calorimeter given by Dr J. F. Counsell. No one concerned with manometry, however experienced he may think himself, could fail to profit by the account of this technique given by Dr Ambrose, who also has some helpful suggestions to offer on fitting experimental results to equations. Dr E. F. G. Herington, in a chapter on "Thermodynamic Quantities, Thermodynamic Data and their Uses", discusses relevant books published in recent years, as well as tabulations of thermodynamic data, and his chapter will therefore be useful to lecturers and teachers in this subject as well as to those who, while not themselves making thermodynamic measurements, use thermodynamic data. Finally, Drs O. Kubaschewski, P. J. Spencer and W. A. Dench, at the end of their chapter on "Metallurgical Thermochemistry at High Temperatures", remark on the challenges presented to the rising generation of chemists by the problems in their field. This remark could be generalized, for no young chemist looking through this book should fail to realize that experimental thermodynamics, so far from being a moribund subject, still offers tremendous scope for human ingenuity. L. A. K. STAVELEY

Chemical Genetics

Molecular Biology: An Introduction to Chemical Genetics. By J. M. Barry and E. M. Barry. Pp. xiii+142. (Prentice-Hall: Englewood, Cliffs, New Jersey, 1973.)

An Introduction to Chemical Genetics is a second, updated edition of a volume in the *Concepts of Modern Biology* series. The material presented covers the development of classical genetics, structure of nucleic acids and proteins, evidence of the chemical nature of the gene, the Watson-Crick hypothesis and its implications, and gene regulation in bacteria.

It seems a pity that with the opportunity to update their book, the authors did not extend their coverage to the problems of eukaryotes, since they comment that "the mechanism of gene regulation in higher organisms is a mystery whose solution is an important task for molecular biologists over the coming years". I would have liked to see some comment on such problems as the stability of messenger RNA in eukaryotes, and the evidence from experiments of Harris using cell fusion techniques regarding the influence of cytoplasmic

components on nuclear activity. Organellar inheritance is now an important feature in cell genetics and some mention of these systems would have been useful.

Concentration on the evidence from bacteria and viruses tends to mislead the student into thinking that all problems in molecular genetics are solved. This book is, however, a suitable introduction to chemical genetics for students starting courses in cell or molecular biology or genetics. It would also be useful for teachers of advanced biology in schools.

SHIRLEY E. HAWKINS

Brain Metabolism

Metabolic Compartmentation in the Brain. Edited by R. Balázs and J. E. Cremer. (Proceedings of a Symposium on Metabolic Compartmentation at the Rockefeller Foundation, Bellagio, Italy, July 1971.) Pp. xii+383. (Macmillan: London and Basingstoke, February 1973.) £9.

THE editors of this symposium and several of its twenty-eight contributors pay tribute to Heinrich Waelsch as giving much stimulus around 1960 to the study of compartmentation in neural systems. Waelsch certainly did so, as part of his larger interests in the structure and functioning of neural systems: a subject which has now so greatly developed that reviews of its current status occupy a five-volume still-growing treatise of Bourne.

To what extent can metabolic compartmentation in the brain be valuably described as a subject in itself? This book suggests the answer—to a limited extent only, though that may not have been the intention of the editors nor is it necessarily my opinion. The book under review begins with a subject central to its titular theme: kinetics of precursor-product relationships, studied isotopically (S. Berl) and in situations which suggest distinct metabolite pools. This is followed by accounts many of which do not deduce, but take as premise, the existence of specified regions; and indeed by accounts, as those of Palay and Glees, which carry few or no metabolic data but describe investigations of structure by orthodox histological and electron-microscopic methods.

Most of the book, as its title specifies, concerns the brain but this does not apply to the group of contributions headed "Intracellular Compartmentation" of which the greater part concerns liver mitochondria; nor to that on "Some Metabolic Characteristics of Organs other than the Brain". Several accounts are given of the distribution of glutamate and its metabolites, and mathematically based models for metabolite distribution are included. Al-

though the book is described as the Proceedings of a meeting "... held to enable a group ... to discuss past and future developments ..." of their subject, little or no discussion among the participants is included, and a major factor linking the disparate parts of the book is its subject index, which is good.

H. MCILWAIN

Battelle Bombast

Science in the Service of Mankind: The Battelle Story. By G. A. W. Boehm and A. Groner. Pp. xiii+132. (Lexington Books, D. C. Heath and Company: Lexington, Massachusetts, and London, 1972.) \$10.

THE title of this book correctly suggests the contents—that is, not so much a history as a house or public relations job. This might not be unusual or blameworthy. It is disturbing, however, that although the Battelle Memorial Institute and its various subsidiary organizations are dedicated to the application of academic standards to practical problems, their president seems not to realize the requirements of professional history. He determined, he tells us, to have "a formal written history" of Battelle and he believes the authors have done "an excellent job". The authors themselves in their foreword are grateful for the opportunity to study the Institute "in depth". Yet the result of so deep a study of "the biggest single operator in American industrial research" is a book of 109 pages (excluding the potted biographies), some of them blank, with wide margins and including drawings. There is not even an index.

In the book, the same president is said to be confident that Battelle will import to its new ventures in contemporary problems a special character derived from its skill in the hard sciences—that is, the quantitative approach which the physicist or engineer applies to everything he does. This approach is singularly lacking in this book; a few figures are indeed scattered about but there is not a single systematic table or systematic quantitative analysis of budgets whether by time, by function, by industrial groupings or by type of customer. The authors claim that they could not fully understand Battelle without also developing a greater comprehension of the interplay of "science, society and the human condition". Alas, they have not conveyed it to the reader. Moreover, it is difficult to have any confidence in factual statements after reading that during the period of expansion in Europe in the 1950s a plan for a laboratory in England was developed "which won the active support of Ramsay MacDonald, then Chancellor of the Exchequer".

The inadequacies of this book are sad,

for there is a great fund of experience in Battelle which is relevant to other organizations trying to perform similar tasks. "As an array of industrial laboratories with more than 5,500 people", we are told, "it is larger than any except the Bell Telephone Laboratories. As a center of science and engineering discovery, it is as active as the University of Illinois. ... As an independent not-for-profit research institute ... it is almost as big and as busy as the next ten combined". Apart from its size Battelle's functions are important. Central to its activities has been contract research, work undertaken for a specific sponsor under a kind of cost-plus arrangement—an idea which seems so eminently sensible when worthwhile separate research departments are so frighteningly expensive, yet one which is so difficult to get off the ground in Britain. Later, Battelle formed a Development Corporation to take ideas nobody else wanted to sponsor and carry them through their high risk period to the point where industry would take them over. The idea is familiar in Britain in the National Research and Development Corporation. The Battelle Corporation hit the jackpot with Xerox which poured cash into its coffers; but this success story is the only real case-study in the book. In all these areas, careful evaluation of all the operations—their costs, results and organization, with the lessons of successes and of the failures—would be most valuable. This unsatisfactory "starter" should be followed by a proper history.

MARGARET GOWING

Fish Farming

Textbook of Fish Culture, Breeding and Cultivation of Fish. By Marcel Huet, in collaboration with J. A. Timmermans. Translated by Henry Kahn. Pp. 436. (Fishing News (Books): London, 1973.) £12.50.

THE fourth edition of Professor Huet's book now appears in an English edition, and is much to be welcomed, though the translation could have been better. The especial merit of the book is in the illustrations, 503 of them, in good half-tone, which may account for the high price asked for the book. But these photographs will convey to an enquirer, who may still have difficulty in finding a going fish farm, though fish culture is expanding, an excellent idea of the layout and equipment of farms for salmonid and cyprinid fish. The scope of this book is wide; and since nowadays fish culture to produce fish for the restocking of angling waters is hardly less profitable than raising fish for food, attention is here given also to the breeding of the pike, pike-perch, walleye, black bass, and many of the so-called

coarse fish, as well as to the many species of trout and salmon of sporting as well as commercial interest.

C. F. HICKLING

Shore Ecology

Life Between Tidemarks on Rocky Shores. By T. A. Stephenson and Anne Stephenson. Pp. xiii+425. (W. H. Freeman: San Francisco, April 1973.) £7.20 cloth; £2.95 paper.

WHEN he died, Professor T. A. Stephenson left the two introductory and the final chapters of a book in which he intended to integrate an unparalleled experience of life on the rocky shores of the world. The present work is largely the result of many subsequent years labour by his widow, Anne, towards this end.

Mrs Stephenson calls the book a salvage operation, and it is for no fault of her own that it falls, perhaps inevitably, between a number of stools. As a contribution to the subject, little new information emerges, although the Stephensons's own work is made more accessible. As a substitute for the unwritten work it lacks the continuity of a single authorship. Its general readability is reduced by a catalogue format, especially since few will recognize personally all the organisms mentioned. Particularly unfortunate is the implication in the title and the earlier part of the text of static zonation caused by tidal phenomena, although the last chapter clearly demolishes such a simplistic assumption. Recent intensive researches in America and Britain have shown that shores are intensely dynamic systems. Distributions change.

The book reiterates a personal view of shore ecology that was unique. T. A. Stephenson was a descriptive naturalist in the old tradition. Here was beauty, magic; the aesthetic too readily dismissed by current objectivism. His descriptions are models of fluency as are his illustrations a delight to the eye.

The publishers have done a great disservice by including so many proof errors, necessitating so large a list of errata. Errors remain: thus on page 274 Garrett should read Gorvett and on page 388 *Balanus tintinnabulum* should read *B. tintinnabulum*. Otherwise the book is neatly produced. Some of the photographs are of indifferent quality, but the book retails at what must be considered today a reasonable price.

Perhaps a commemorative volume contributed by workers presently in the field would have been a more vital memorial, thereby acknowledging our debt to the Stephensons whilst reminding readers that times have changed. The definitive work on shore ecology still remains unwritten.

P. G. MOORE

CORRESPONDENCE

Price of Books

SIR,— You recently asked me to review a book for *Nature*. I was tempted to remark in the review that the book was very expensive. Then it occurred to me that nothing happens when reviews include such perfunctory remarks, and that anyway most scientific books are very expensive now. This led to wider questions. Do scientific authors, publishers, printers or booksellers make more money than they deserve at the expense of the scientific community? Does the system of advertising, whereby piles of letters reach me each month, add a lot to the prices of books? Should we scientists organize ourselves to prevent our exploitation by others? We grumble about the cost of books but do nothing. It is tempting to blame the publishers, but I, for one, would not deny them a fair profit, even if they may have subsidized the scientific community in the past.

It so happens that the book I reviewed was one of a series. (I think the name of the publishers is unimportant, because their prices do not seem unusual.) So I have plotted the year against the

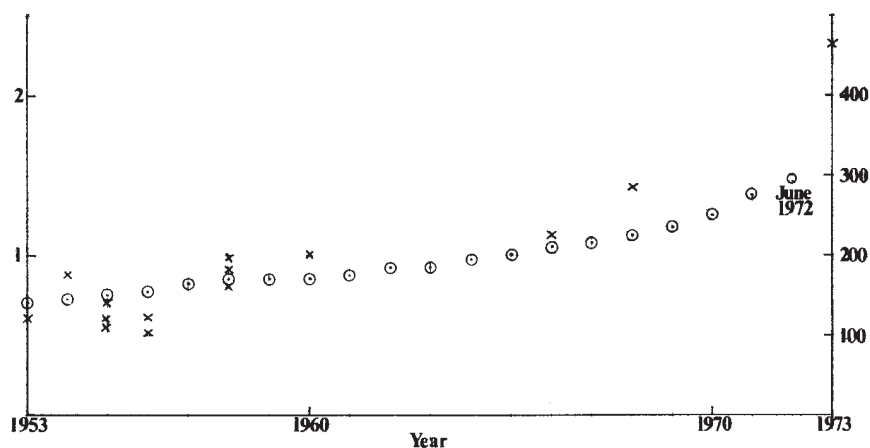


Fig. 1 Rise in book prices: x, cost (£) per 100 pages; o, index of retail prices (all items, 1947=100).

publication price per 100 pages of each book of the series and against the index of retail prices—all items (Fig. 1). One can dispute the implication that this affords a fair comparison between what a book should cost and what it does, but nonetheless I think two conclusions are clear: the costs of living and of books were fairly consistent until the end of the sixties; rampant inflation

thereafter has affected books somewhat more than the cost of living. Should we organize a lobby, or be grateful that things are not worse than they are?

Yours faithfully,

P. G. DRAZIN

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Obituary

Richard J. Winzler

GLYCOPROTEIN research, cancer research and biomedical education suffered a severe loss with the sudden death of Richard J. Winzler on September 28, 1972.

Winzler was born in San Francisco on September 29, 1914. He received his early education in the San Francisco area and obtained his PhD from Stanford University in 1938, at the age of 23. Following this he was Sterling Fellow at Yale University (1938–1939) and National Research Council Fellow at the Wenner Gren Institute, Stockholm (1940–41). On the outbreak of the Second World War, Winzler returned to the USA to complete his NRC fellowship at the National Cancer Institute, Bethesda (1941–43). During this early period his interests began to change from considerations of metabolic reactions in normal cells, especially yeast, to studies of the biochemical changes which differentiate normal and malignant tissues.

Following these *Wanderjahren*,

Winzler was appointed Assistant Professor at the University of Southern California at Los Angeles in 1943 and rose through Associate Professor to Professor in 1949. In 1952 he was appointed Chairman of the Department of Biochemistry at the University of Illinois College of Medicine.

Throughout this period of increasing burdens in teaching and administration, Winzler maintained an active role in research, continuing his studies on normal and abnormal metabolism, including the role of thyroxine, but with a developing emphasis in the field of glycoproteins in which he was to make his major contributions. In particular, he recognized the widespread distribution of glycoproteins in serum, developed methods for their isolation and characterization, and began studies on their structure and biosynthesis. He realized that sialic acid was a major determinant of glycoprotein structure and that the glycoprotein levels varied in certain pathological states. It is from this early work that some of our recent understanding on the role of carbo-

hydrate in glycoprotein function can be traced. At the same time Winzler continued his studies on biochemical changes in neoplastic tissue and extended these to studies of the biochemical effects of viral infections.

In 1965 Winzler accepted the position of Chairman and Professor at the Department of Biochemistry, School of Medicine, at the State University of New York at Buffalo. Despite the administrative burdens of extended planning for new medical, dental and research facilities this was probably the most productive period of his life, as his wide knowledge of glycoproteins became focused on the glycoproteins of cell membranes. A significant step forward in this area was made by his isolation of the major glycoproteins of the red cell membrane, their structural characterization and the elucidation of monosaccharide sequence in certain of their subunits. This led him to the development of a model for membrane glycoprotein structure, identical with that proposed at about the same time by Morawieki, in which the hetero-

saccharide subunits are clustered toward one end of the polypeptide chain while the other end contains predominantly lipophilic residues serving to anchor the glycoprotein in the membrane. Subsequent studies have supported this model in erythrocytes and other cells.

In 1969 Winzler decided to free himself of the onerous administrative responsibilities of the chairmanship at Buffalo and, in order to devote more time to teaching and research, accepted a Professorship in Chemistry at Florida State University under the auspices of a National Science Foundation Development Grant. He entered into this new position with characteristic enthusiasm and was beginning to establish his research group with an increasingly strong emphasis towards the biosynthesis structure and function of the glycoprotein components of cell surfaces. This work was not completed.

Winzler was a Visiting Professor at the University of Wisconsin (1941), a Commonwealth Fellow at the University of Freiburg (1958) and a Consultant in Medical Education at the University of Chengmai, Thailand (1962). He served on the Editorial Boards of *Cancer Research* and of the *Proceedings of the Society of Experimental Biology and Medicine*, on several policy committees of the American Cancer Society and the National Institutes of Health, and on the Advisory Committee of the American Red Cross National Fractionation Center. One of his last contributions to determining future direction in biomedical research was in serving on the Planning Committee for the development of the Program Plan of the National Cancer Institute when it was elevated to the rank of Bureau with the National Institutes of Health.

Winzler could work in a laboratory with the enthusiasm and dedication of a graduate student. For a period of two weeks he worked in the writer's laboratory on the isolation of red cell ghosts in large amounts for his structural studies on the M and N antigens. He would work until late at night and cheerfully signed the receipts required by our supplier for the large purchases of sugar needed for agglomeration and washing to assure him that they were not being used for illicit fermentation.

Dick Winzler made major contributions in diverse areas of research, in the teaching of medical and dental students and scientists and in the administration and development of several medical schools and universities. He was widely respected as a teacher, a scientist and a friend and, with the approval of his wife, Janna, and their three children a memorial fund has been set up at Florida State University to promote an interest in biochemical research.

Announcements

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

- Incorporated Society for Psychical Research. Annual Report of the Council and Statement of Accounts, 1 October 1971-30 September 1972. Pp. xxi. (London: Incorporated Society for Psychical Research, 1 Adam and Eve Mews, W8, 1973.) [263]
- Cosmatom*, Vol. 1, No. 1, February 1973. (A Journal devoted to the Study of Five-Dimensional Space-Time-Mass, and to Consideration of the View that the Cosmos and the Atom are Mutually Explanatory.) Pp. 1-68. (Worthing, Sussex: *Cosmatom*, P.O. Box 12, 1973.) £1. [263]
- Medical Research Council Handbook 1972-1973. Pp. iv+268. (London: Medical Research Council, 1972.) £1. [273]

- Local Government Boundary Commission for England. Report No. 2. Pp. iii+9. (London: HMSO, 1973.) 16p net. [273]
- Industrial Research and Development: The Report of the Committee of Enquiry into the Research Associations, 1972/3. Pp. 195. (London: Conference of Industrial Research Associations, 29/30 St. James Street, SW1, 1973.) £3. [283]
- A Computer Simulation Model for Bus Operations Planning. By J. P. D. Gerrard and D. Brook. Pp. 38. (Peterlee, County Durham: UK Scientific Centre, IBM United Kingdom Limited, 1972.) [283]
- Albright and Wilson, Ltd. Annual Report for 1972. Pp. 28. (London: Albright and Wilson, Ltd., 1973.) [293]
- Southampton University Library. Occasional Paper No. 4: The Literature of Science and Technology Approached Historically. By Dr Raymond V. Turley. Pp. 24. (Southampton: The University, 1973.) 60p. [293]
- Bulletin of the British Museum (Natural History). Entomology. Vol. 27, No. 8: A Revision of the *Lecanodiaspis* Targioni-Tozzetti (Homoptera: Coccoidea) of the Ethiopian Region. By C. J. Hodgson. Pp. 411-452. £1.75. Vol. 28, No. 2: G. B. Buckton's Works on Aphidoidea (Homoptera) By J. P. Doneaster. Pp. 23-109+6 plates. £3.55. Supplement 19: A Revisionary Classification of the Rutillini (Diptera: Tachinidae), with Keys to the Described Species. By Roger Ward Crosskey. Pp. 167. £6.50. Zoology. Vol. 23, No. 5: The Gunong Benom Expedition 1967. 6. The Distribution and Altitudinal Zonation of Birds and Mammals on Gunong Benom. By Lord Medway. Pp. 103-154. £2.05. Vol. 24, No. 4: Spirobinae (Serpulidae: Polychaeta) from South-Eastern Australia. A New Genus, Four New Subgenera and Seven New Species. By Phyllis Knight-Jones. Pp. 229-259. £1.25. Vol. 24, No. 5: On the Cyprinid Fish *Barbus Allaudi* Pellegrin: a Possible Intergeneric Hybrid from Africa. Studies on African Cyprinidae, Part 1. By K. E. Banister. Pp. 261-290+1 plate. £1.45. Vol. 24, No. 6: The Cyprinid Fishes of *Acanthobrama* Heckel and Related Genera. By M. Goren, L. Fishelson and E. Trewavas. Pp. 291-315. £1.15. Supplement 4: Cardigan Bay, Recent Foraminifera (Cruises of the R.V. Antur, 1962-1964). By J. R. Haynes. Pp. 245+33 plates. £10.80. (London: British Museum (Natural History), 1972 and 1973.) [293]
- Innovation and the Academic Engineer. By Professor Hans Moitz. (An Inaugural Lecture delivered before the University of Oxford on 21 November 1972.) Pp. 25. (Oxford: Clarendon Press; London: Oxford University Press, 1973.) 50p net. [303]
- The Child with Cerebral Palsy. Social, Emotional and Educational Adjustment—an Annotated Bibliography. By Doria Pilling. Pp. 61. (London: National Children's Bureau, 1 Fitzroy Square, W1, 1973.) £1.25. [303]

Other Countries

- The University of Adelaide. Centre for Precambrian Research. Special Paper No. 1: Stratigraphic Problems of the Later Precambrian and Early Cambrian. Edited by J. B. Jones and B. McGowan. Pp. iii+70. (Adelaide: University of Adelaide, Centre for Precambrian Research, 1972.) \$A2; \$US2.50; £1. [222]
- Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIV, No. 4: Numerical Methods for Computing Stellar Line-Profiles and Continuum Fluxes. By J. B. Hutchings. Pp. 59-96. Vol. XIV, No. 5: The Spectroscopic Binary System H.D.11860. By A. H. Batten and B. Szeidl. Pp. 97-105. (Victoria, BC: Dominion Astrophysical Observatory, 1972.) [222]

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